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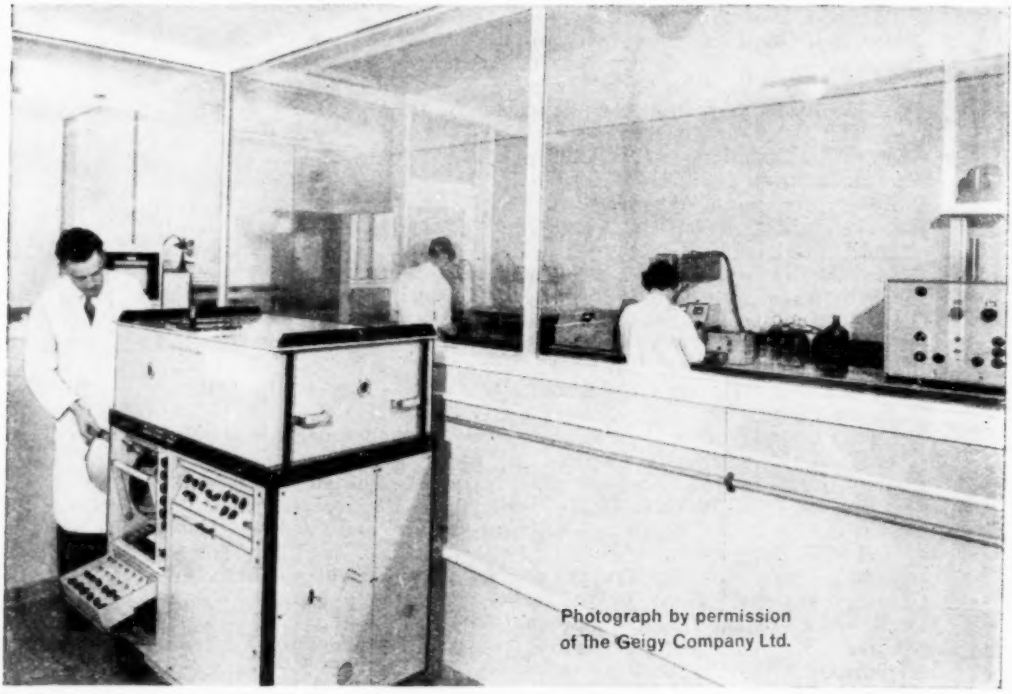

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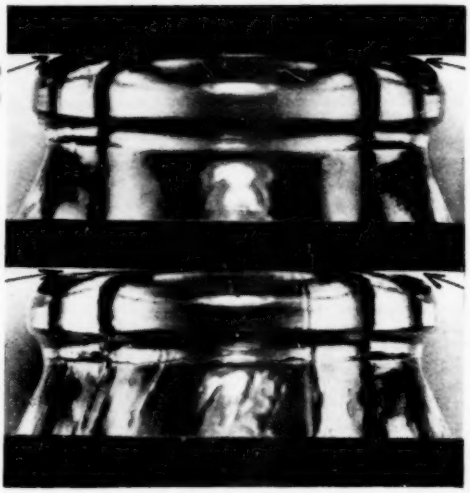

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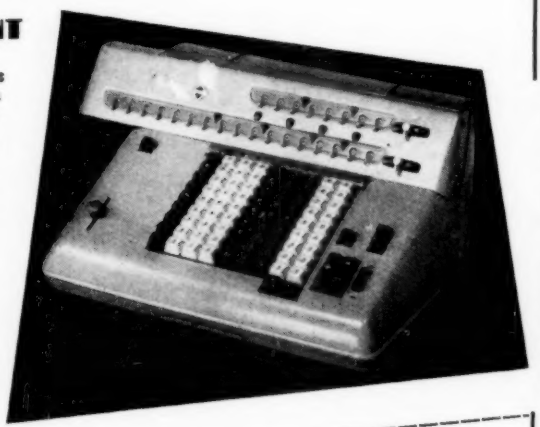

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THE NATIONAL REFERENCE LIBRARY FOR SCIENCE AND TECHNOLOGY

ABOUT a year ago, on May 31, 1960, the Minister for Science, in reply to a question in the House of Lords, announced that the National Reference Library of Science and Invention, which he had already stated on July 30, 1958, was to be housed in a new Patent Office building to be erected on the South Bank in London, is to be formed as part of the British Museum Library; it was to be housed as a separate unit, and scientific publications at present in the British Museum Library would be incorporated in the new Library. Lord Hailsham then said that the terms of a lease of the site were being settled between the Ministry of Works and the London County Council, and that it was hoped to start building in the spring of 1963, with the view of completion by the end of 1965. Some further information was supplied by the Parliamentary Secretary for Science, Mr. Denzil Freeth, in replying for the Government in an adjournment debate on the subject on March 14, 1961; further, the Association of Special Libraries and Information, in view of the importance of the issues involved, has devoted almost the whole of the March 1961 issue of the *Journal of Documentation* to a symposium of important papers on the subject.

In the first of these, Prof. R. S. Hutton, writing with the authority of his experience on the Council of the Association of Special Libraries and Information Bureaux, and as a user of the Patent Office Library for nearly sixty years, reviews the contribution which that Library has made to the needs of the scientist and technologist, its decline in standards since 1914, and the official procrastination both before and after the Second World War. The difficulties and problems which any such library must face are fairly though briefly stated, but Prof. Hutton lays the responsibility for the present situation unequivocably where it belongs—on the shoulders of successive Governments which have refused to face the issues clearly presented to them by their own advisers. In the second paper, Mr. E. M. Nicholson, director-general of the Nature Conservancy and chairman of the Scientific Library and Technical Information Committee of the Advisory Council on Scientific Policy, reviews in greater detail the steps which, after the abandonment of the proposal for a 'science centre' in London, issued in the present project. He is no more inhibited than Prof. Hutton in his criticism of Government delays, and it is significant that, in welcoming the physical link of the new library with the Patent Office in occupying the same building, he gives as one reason the fact that the London County Council has made it a condition of providing this site that building should begin in the near future. This, Mr. Nicholson quietly remarks, provides the best possible safeguard against a continuation of procrastination in getting on with the job. For all the lip service which Governments of both political parties have paid to the need to support scientific and technical research and its application, and to expand the number of scientists and technologists, it is, in fact, difficult to parallel the record of neglect, indifference and waste of public money and talent which has characterized official policy in this field during the past fifteen years.

A second reason which Mr. Nicholson gives for welcoming the new site is its proximity with a great complex of offices of one of the largest industrial concerns in Europe. This, he rightly points out, should make contact with users easier. Further, he suggests that had adequate contact been maintained between scientific libraries and their users, these libraries would not, alone among scientific institutions, have failed to benefit by the greatly increased resources recently made available for science. At the top level, this lack of contact has meant that even the high prestige and assiduous efforts of the Advisory Council on Scientific Policy have barely been able to neutralize the apathy and inertia which beset almost every effort to secure greater resources for the national institutions of Britain in the library field.

That is putting it generously, for not every informed observer would concede as much. Moreover, Mr. Nicholson emphasizes that information and advice have had in consequence to be obtained from a much more limited range of sources than would be expected in view of the number of interests and institutions potentially concerned; and he suggests that there is virtually no body of senior people professionally trained both as scientists and librarians who could form a source of man-power. That proposition is more open to debate, however, though admittedly on any definition there are few enough such senior people. Responsibility, however, lies to some extent with the profession itself: the perennial discussions on training intelligence or information officers and librarians have not always been either so profitable or constructive as they might have been.

Whether the work that has gone into the launching of the National Lending Library for Science and Technology will be of some benefit to the Reference Library also remains to be seen; but Mr. Nicholson is possibly a little optimistic in looking to the advisory committee under Sir Lindor Brown's chairmanship. It is precisely in respect of the representation of industrial users that the composition of that committee has been criticized. Prof. Hutton, who equally stresses the importance of the users expressing their point of view on the full-scale needs of these libraries, looks rather to the Association of Special Libraries and Information Bureaux, and urges that advisory committees for each library, and opportunities for those who use the libraries to voice their needs, are most desirable. Mr. Freeth, on March 14, explicitly

committed the Government to the establishment of an advisory committee for the new library.

Mr. Nicholson lays some stress on the recruitment of suitably qualified scientific staff with adequate competen e in librarianship, and is disturbed that recruitment should seriously be contemplated of librarians who know no science or scientists with no training in librarianship. On this point there has been some criticism of the policy adopted for the National Lending Library, on which Dr. D. J. Urquhart does not appear to have entirely satisfied his critics. On Mr. Nicholson's further point that, in organizing the accessibility and communication of this vast harvest of scientific research, we should be virtually devoid of research results and advice in the provision and organization of scientific libraries, some reassurance is to be found in Mr. Freeth's reply on March 14. The Government is already considering how best research into information techniques can be promoted, and it is fully agreed that the new Library should co-operate in this kind of research in any way in which it may be able.

In the meantime, however, the National Science Foundation in the United States is very actively promoting such investigations, and a touchstone of the Government's sincerity in this particular respect may well be found in the extent to which the Association of Special Libraries and Information Bureaux is enabled to undertake similar investigations. This, of course, is not to deny the importance, recognized by Mr. Freeth, of sound judgment in the selection of projects. The formulation and selection of projects, moreover, need not await the coming into operation of the new Library, and, as Mr. Nicholson's paper sufficiently indicates, there are many matters on which we are as yet too inadequately informed for sound decisions as to the equipment and operation of a first-class modern scientific library. That could well be totally different from libraries as even many librarians now visualize them.

In view of the importance of co-operation between user and library, the remaining two papers in the symposium are of special interest. Miss Maysie Webb, who in April 1960 was appointed head of the Patent Office Library with this preparatory work in mind, reviews the pattern of usage of the present Library and the plans for the future; and Mr. A. H. Chaplin, keeper in the Department of Printed Books, British Museum, to which the new reference Library will be attached as a division, considers some of the problems arising out of that relation. Mr. Freeth, on March 14, also referred to the work being done on the internal arrangement of the new Library by Mr. Wilson, of that Department, and by Sir Frank Francis, director of the British Museum, who himself is a member of the library committee of the Advisory Council for Scientific Policy. He rejected for the present Mr. Austen Albu's suggestion of a special advisory committee, and recommended that suggestions which scientists, industrialists, librarians or others may have for the new Library should be sent direct to the people he had named as concerned with its planning.

Miss Webb begins by directing attention to three broad principles on which the operation of the Patent Office Library has been based in the past. First, it has been recognized that in an economy increasingly geared to science and technology, it is prudent to make freely available a store of such knowledge, thus providing the scientist with his raw materials, stimulating invention and facilitating the work of the technical community in general. This outlook is reflected in the 'open-access' facilities, and Miss Webb states explicitly that the whole of the new South Bank library premises will be designed with open access as a basic requirement. This facility has been a most valuable feature of the Patent Office Library; while Miss Webb also indicates that the Library staff will play a more active part in exploiting the resources of the new Library and in helping users at all levels, her paper will be warmly welcomed by scientists and technologists for this firm assurance alone, with its corollary that seating accommodation for at least half the expected number of readers, close to or among the bookshelves, is to be provided.

Miss Webb, moreover, is well aware of the frustration which can arise from the lack of immediacy in a closed-access system, and there is already some uneasiness on this score in view of the fact that this new reference library is to be associated with the British Museum. It was recently pointed out in the House of Commons that, while every user of the British Museum Reading Room greatly appreciates the efficiency, courtesy and ability of the staff, it still takes nearly an hour and a half to obtain a book compared with 12 or 15 min. at the Bibliothèque Nationale in Paris. Miss Webb's paper offers some assurance on this point, and it is evident, too, from Mr. Chaplin's paper that the importance of the time factor in the new arrangements is fully appreciated on the British Museum side. Although many administrative questions have still to be resolved, the final arrangements will be such as to support fully the open access principle and provide rapid and frequent transport between the two libraries for handling the marginal material or older material of only occasional usefulness transferred to the British Museum itself.

The third principle emphasized by Miss Webb is that the Patent Office Library has been concerned in the past with the field of invention, catering in general for the graduate or practising scientist. The new library will broaden the range and thus alter the balance, and in discussing plans for the future Miss Webb refers to the possibility of increasing the depth as well as the range of subjects covered, particularly in respect of material in foreign languages, technical trade literature and translations. It is also hoped that much extra material will be available as a result of the link with the British Museum, though legislation will be needed before this material could be available for consultation at short notice.

This aspect is discussed more fully by Mr. Chaplin, who visualizes the British Museum playing an important part in research in the history of science, provided the legislation referred to by Miss Webb is enacted and that the British Museum is assured

of sufficient storage space to receive the material discarded by the Reference Library. Moreover, while he emphasizes the need for rapid and frequent transport between the two Libraries, he recognizes that there are other means of meeting urgent demands which require attention; such devices as the telegraphic transmission of photo-copies or closed-circuit television may be instanced. Like Miss Webb, Mr. Chaplin recognizes that documentation activities should have an important place in the programme of the new library, and he visualizes this as a more specialized extension of work already undertaken by, or in close association with, the British Museum. Miss Webb also referred to the preparation of subject bibliographies and selective reading lists, and it is clear that she contemplates that the new Reference Library will be staffed so as to admit of such activities to a certain extent.

How far such activities will materialize remains to be seen. At present only two of the staff of the Patent Office Library are science graduates and nine professional librarians. While it is not expected that specialists in all the subjects to be covered will be recruited, even in the planning period of the next few years it would seem that there is room for a good deal of improvement in the proportion of academic and professional staff. Miss Webb appears to be well aware of the problem, however, and to recognize that such staff is needed long before the new Library begins to operate.

Meanwhile, Miss Webb puts the emphasis on a detailed research programme, and it should be noted that she looks to find some of the answers to questions about the new Library and its readership in co-operation with its users. The Advisory Council on Scientific Policy has already recommended that research into the whole problem of collation and dissemination of scientific information should be undertaken under the auspices of the new National Lending and Reference Libraries for Science and Technology, and Miss Webb recognizes that, given all the co-operation of users, this work should be proceeding already and requires the recruitment of staff for the purpose.

On that point Mr. Freeth was less reassuring than he might have been. He agreed that the new Library needed a sufficient staff with academic training capable of extending their fields of knowledge in a scientific direction, and that it should have an expert staff capable of providing a first-rate information service for those using the library. But he appeared to think that the matter of research into information techniques and the like could be left until the new Library is operating. As already indicated, this will be a test of the reality of the Government's interest in the matter, and in this connexion the concluding paragraph of Mr. Nicholson's paper should be considered.

"Let us hope", he wrote, "that those who now have to put into execution this vital project will succeed in realizing their opportunities to the full. Let us also remember that until we see the National Science Reference Library opened we can never feel actively confident that there will not be some other slip between the cup and the lip. Anyone who may be tempted to jog the arm that lifts the cup should, therefore, think twice about it." From one who has been so closely associated with the Scientific Library and Technical Information Committee of the Advisory Council on Scientific Policy, such a caution is the more significant. It is clear that Miss Webb and those associated with her in planning the new Library and its services have sensible views of its functions and possibilities, that they are aware of the potentialities of new techniques, as well as of the solid advantages of some practices of the past, and that they are anxious to secure the co-operation of all potential users. Such co-operation should be given generously and imaginatively, but more than constructive criticism is required. Let it not be forgotten that Government parsimony and short-sightedness could yet once again jettison the whole scheme, that procrastination in dealing with needs as they arise could multiply costs once again out of all proportion and destroy efficiency. It is for users, therefore, to co-operate also in seeing that at all levels the Government is kept aware of the magnitude and urgency of the problem, and the needs of the new Library are clearly and continuously presented throughout the planning period and when it is fully operational. In that task not only the individual scientists and technologist but also their professional associations, and such bodies as the Association of Special Libraries and Information Bureaux, the Parliamentary and Scientific Committee and the Advisory Council for Scientific Policy, have a responsibility which should be vigilantly and resolutely discharged.

EXPLOSIVES TECHNOLOGY

Solid Propellent and Exothermic Compositions.

By Dr. James Taylor. (Newnes Technical Surveys). Pp. vii+153+12 plates. (London: George Newnes, Ltd., 1959). 25s. net.

THIS book provides a very useful and interesting survey of the development of exothermic chemical reactions in the solid state as sources of energy for many applications and devices. It contains broad reviews of modern developments in initiators, explosives and propellants. The author, Dr. James Taylor, has spent the greater part of his working life in the explosives industry; he has made many personal contributions to the developments described, and has drawn freely on the work of his colleagues in the Nobel Division of Imperial Chemical Industries, Ltd.

There is a chapter devoted to the history of gunpowder, its applications as a propellant and its present-day use in ignition and delay systems, fireworks and military pyrotechnics. Explosives are rather briefly reviewed—the author dealt comprehensively with the performance of commercial blasting explosives in his monograph *Detonation in Condensed Explosives* (1950). There is a description of the various types of initiating explosives (detonators, fuse-heads and percussion primers) and the modern types of propellants used in small arms and

guns are reviewed; this is followed by an interesting account of the gas-producing reactions that have been used in devices to improve the safety of blasting operations. The chapter on rocket motors is particularly well written, and there is an extremely good review of "power cartridges", starting with the "humane cattle killer", which was developed just after the First World War, and leading up to the numerous devices which have been developed in the past thirty years for operations such as starting aircraft engines, inflating life-jackets, propelling model aircraft, etc. All these applications call for a high level of reliability and this has been achieved in spite of the complexity of many of the systems. In each of the fields covered, the thermochemical and ballistic aspects are very adequately dealt with for a proper understanding of the design principles and working of the applications described.

It is, of course, unfortunate that the contributions by Government research organizations towards many of the developments described cannot be dealt with because much of the subject-matter is of a classified nature and this leads, as the author points out in the preface, to some lack of balance in the over-all treatment. However, the book achieves its aim and is warmly commended to those seeking an introduction to branches of explosives technology that have greatly increased in importance in recent years.

W. B. Littler

SOME BACTERIAL PLANT DISEASES OF CENTRAL EUROPE

Bacterial Plant Pathogens

By C. Stapp. Translated by A. Schoenfeld. Pp. xviii+292. (London: Oxford University Press, 1961.) 42*s*. net.

During the past sixty years the knowledge of plant diseases has been enlarged at an ever-increasing rate, mainly due to the careful investigations of German plant pathologists, which were collected together by Paul Sorauer and appeared in 1928 in the third edition of his *Handbuch der Pflanzenkrankheiten*. As new knowledge accumulated, larger editions were called for, and in each one Dr. C. Stapp contributed the section dealing with bacterial diseases of plants. In the 6th edition, issued in 1956, the material to be dealt with was so great that Dr. Stapp's account was published as a separate volume of the *Handbuch* entitled *Bakterielle Krankheiten*. This great work by so well-known and so highly esteemed an investigator contained accounts of, and references to, all recorded diseases up to 1955 and was admirably illustrated.

The advances in this subject made in the past ten years, the great interest shown by both university and high-school teachers, and the prohibitive price of his *Bakterielle Krankheiten* induced Dr. Stapp in 1958 to bring out a less-expensive book based largely on his previous comprehensive work. It also included accounts of some twenty-four diseases occurring in Europe, with references up to 1957. It was printed on highly glazed white paper on which the numerous photographic illustrations were clearly reproduced and it concluded with a very complete index. The title was *Pflanzenpathogene Bakterien*.

Now, in 1961, this admirable work has been rendered into English because, as Dr. Stapp indicates in the English preface (also dated 1958), besides the rising interest in Germany, a like desire for information on the bacterial diseases of plants has manifested itself in "other Central and Western European countries and also in some countries outside Europe". It is certainly flattering to know that plant pathologists of these "other" countries apparently prefer English to German. The translator has produced an elegant, though not always accurate, rendering of Dr. Stapp's German and includes his very few errors such as *Pseudomonas delphinii* being pathogenic only to the Cruciferae (Stapp, p. 12, Schoenfeld, p. XII) and *Prunus devaricata* for *P. divaricata* (Stapp, p. 177, Schoenfeld, p. 188).

The English version follows exactly the arrangement of the German edition, the chapters, headings and sectional paragraphs are in the same order, and, as in the original, there are two parts, a general part concerned with methods and identification and a special part describing in detail some twenty-four plant diseases. Both parts are equally instructive. In the first we have Dr. Stapp's own bacteriological methods, some of which are rather unusual. He prefers steaming to autoclaving, neutralizes his media either with sodium carbonate or with dilute phosphoric acid and stains his smears on cover-slips, not on slides. The method, which is illustrated, of making transfers of pure cultures whereby both tubes and their extracted plugs are manœuvred into the same hand is not a common practice in Britain nor is it universal in Germany. A slight error occurs in both the German and English editions in the description of sterilizing Crosse and Bennett's selective medium in which the authors state (*Trans. Brit. Myc. Soc.*, 38, 83; 1955) "and sterilized by bringing the pressure slowly (3–5 min.) up to 7 lb." Dr. Stapp's version (p. 26) is "Druck langsam auf 1 atü [that is, 1 atmosphere which equals 15 lb. not 7] bringen und darauf 3–4 Minuten halten". In the reference the abbreviation *Trans.* (*Transactions*) is omitted in both editions (Schoenfeld, p. 10).

In the special part the descriptions of the diseases and their pathogens vary in length, that dealing with crown gall (*Agrobacterium tumefaciens*) being the longest, and containing clear accounts of the various theories of tumour-formation. In the chapter on black-leg of potato nearly 5 pages are devoted to Dr. Stapp's arguments for naming the pathogen *Erwinia phytophthora* (Appel) Holland in preference to *E. atroseptica* (van Hall) Jennison. Of most interest perhaps at the moment is the chapter on fireblight caused by *E. amylovora*, which has only recently been recognized in southern England. The account of plum and cherry canker caused by *Pseudomonas mors-prunorum* (spelt in one word—*morsprunorum*) is thoroughly up to date; the wilt of beans due to *Corynebacterium flaccumfaciens* is also included although it does not occur in Germany.

Some of the phrases used by the translator are apt to be misleading and some are quite inaccurate. As examples, on p. 2 we read about "Koch's steam-pot", a literal translation of "Koch'schen Dampftopf" (p. 19), meaning steam sterilizer or just steamer: on p. 3 is "if the nutrient medium is not bottled immediately", but Dr. Stapp writes (p. 19) "nicht gleich in Röhrchen [that is, tubes] abgefüllt": on p. 4, "The meat infusion is measured and weighed", whereas Dr. Stapp would do one or the other (p. 21, "abgemessen oder abgewogen"). On p. 16 is "breed pathogens from" for isolate in pure culture (German p. 31 "reinzuzüchten") and in the footnote on the

same page we find "incubated . . . for the first time" for isolated . . . for the first time (German p. 32, "erstmalig . . . herausgesüchtet"). On the same page occurs "Koch's dishes" for the German (p. 31) "Koch'schen Platterverdünnungen" (that is, Koch's dilution plates). On p. 19 "woodland fruits" is the translation of "Obstgehölze" meaning fruit trees, and later on the same page is "the inserted graft is covered with phloem tissue" for the German (p. 34) "Bast" meaning bast fibre (used by horticulturists).

At the end of section 6 this occurs—"every biologist should acquire during his working life", which is not quite the same as Dr. Stapp's statement (p. 35) "jedem Biologen eigen sein sollte" which might be better rendered—every biologist should naturally possess.

In many places when describing the action of bacteria in milk the German word "peptonisiert" becomes "peptone formed", which may be biochemically correct, but the more common English term would be peptonized or digested or even cleared. On p. 21 the German (p. 37) "auf der letzten Platte" (on the last plate) is rendered "at the bottom of the last plate" and on p. 22 we find the German (p. 37) "rechtwinklig" (right angle) becomes "rectangle"! On p. 55 it is actually stated that "freshly diseased blackleg potato shoots are easily obtainable from the fields throughout the year . . ." an utter impossibility! But Dr. Stapp writes "alljährlich", that is, every year.

Apart from incorrect and misleading statements it would seem that the translator is not too well acquainted with English bacteriological terminology.

A few printing errors have been noted, for example, on p. iv "bakterian" appears for "bakterien" and on p. 146 "See p. 9" should be "See p. 17", p. 236 is followed by p. 137 and in Table 4 on the same pages, *Nicotiana lustica* should be *N. rustica*.

The book concludes with a very comprehensive index after Dr. Stapp's style, but a misprint occurs on p. 286 where *Pseudomonas populans* is printed for *P. papulans*. The numerous references in the text do not all appear at the end of each chapter and for the missing ones recourse has to be made to Dr. Stapp's large volume *Bakterielle Krankheiten*.

Although beautifully produced, the illustrations are not as clear as those of Paul Parey, probably owing to the less highly glazed surface of the cream-coloured paper.

This is not a book which may be recommended for the inexperienced worker as his only source of information.

W. J. Dowson

AN APPRAISAL OF PHENOMENOLOGY

The Phenomenological Movement

A Historical Introduction. By Dr. Herbert Spiegelberg. (Phænomenologica, Vols. 5 and 6.) Vol. 1: Pp. xxxii + 392. Vol. 2: Pp. ix + 393–735. (The Hague: Martinus Nijhoff, 1960.) 47.50 guilders the two volumes.

THESE two volumes, erudite and well produced, set out to offer a historical introduction to that branch of philosophy commonly known as the phenomenological movement. Its chief exponent, Edmund Husserl (1859–1938), would have preferred some such terms as 'circle' to 'movement', but to English-speaking students the difference is immaterial. In fact, the author is at pains to present his work in a form most helpful to readers in the United States: they seem to need a bridge to span the gap between their own intellectual climate and that of Europe. Not that they lack competent exponents; yet the background is very different from what is basically an admixture of *Gestalt* psychology and *Geisteswissenshaften*. Biographers need no reminding that Husserl started as a pupil of the great Weierstrass, and therefore cannot be said to have been devoid of mathematical sensitivity. It is probably true, however, that in claiming phenomenology as fundamental and prior to all scientific thinking he gave an impression of detachment from techniques which might, in a more narrow sense, be said to belong to the natural sciences as we know them. Nevertheless, "back to the things themselves" is not a bad imperative for any laboratory, especially when to it is added the need to orientate ourselves towards phenomena in front of which theoretical patterns have become too obtrusive. That, in brief, is the phenomenologist's aim. Several schools of thought have wrestled with this problem before, but the present attempt is focused more sharply.

Although somewhat negative, one important characteristic of the *Methodik* must be mentioned. It is the critical outlook which emerges in relation to Occam's razor. Probably few devices have been more misconceived and misconstrued. Simplicity and parsimony are all very well as disciplines, but the former is to be distrusted if it means little else than an artificial scraping to the bone of our concepts, and the latter when it leads to a degree of abstraction utterly at variance with the true pattern of Nature. Incidentally, this is why the razor is not the correct tool for—say—rheology and morphology. Here, odd as it may seem, parameters and constants can well be introduced liberally, even if later on their number can be substantially reduced and their scope narrowed. As Dr. Spiegelberg remarks, reverence and the lens are more fitting instruments than a bulldozer. The age-long parable of the wheat and the tares is directly applicable—"Let both grow together until the harvest".

All of which is but to recognize that every type of perception should be given a chance, including even those not necessarily involving the presence of a sense-organ for their registration. Of course, the eye, as the window of the mind, remains paramount, but *gestalten* other than visual are known, and are fully worthy of a place.

The 'feel' of these pages has been deliberately presented in advance of a factual statement of their content. This is because there are almost as many phenomenologies as there are phenomenologists, and to expect a crisp formula describing the labours of the latter would be to ask the impossible. Nevertheless, by a sound system of classification of his material, the author prevents the reader from straying too far from the historical track.

Thus, after some introductory material, the first part deals with the preparatory phase; Part 2 with the German phase. Then comes a very accomplished 'lead-in' as to how and why the movement spread to France (Part 3), where it achieved considerable support and success. Which done, we are asked in Part 4 to assess the position at mid-century, and incidentally the cool reception of the subject in Great Britain stands out clearly. Finally, in Part 5, principles and appraisals give the inquirer as good an

idea (outlined in the first part of this review) of what it is all about, as can be required of anybody reporting on so difficult a theme.

Lastly, there are several pages of charts surveying the development of phenomenology in Germany and in France, followed by indexes and a glossary.

All this is a most valuable addition to the literature of the subject. One could make minor criticisms of style, like the irritating repetition of the phrase "to be sure" and the graceless abbreviation "photo". But these are trifles in an excellent treatment. Edmund Husserl remains at the centre of the stage, around him grouped both disciples and critics united, whatever their differences, to do him honour.

F. I. G. Rawlins

MODERN STYLE IN MATHEMATICAL ANALYSIS

Foundations of Modern Analysis

By J. Dieudonné. (Pure and Applied Mathematics: a Series of Monographs and Textbooks, Vol. 10.) Pp. xiv+361. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 8.50 dollars.

In recent decades tremendous changes have come about in many branches of pure mathematics. Not only have content and emphasis altered, but in addition there has been a fundamental change in the interpretation of large sections of classical mathematics. Abstraction and generalization, leading to the unification of previously scattered concepts, have played a powerful part in this process. One of the most vigorous protagonists of this activity has been the Bourbaki school of French mathematicians. The Bourbaki point of view on mathematical analysis is admirably presented in the book by Prof. J. Dieudonné. For every notion the concept itself is emphasized rather than the manipulation involved in using the notion, and this applies to the large number of problems as well as the text itself.

The book starts with an account of the theory of sets and the properties of real numbers and the real line. Then come two very important chapters, on metric spaces and normed spaces. The contents of these chapters form the backbone of the whole work, and are fundamental for all that follows. The important notions in a metric space are completeness, compactness and connectedness. These are carefully introduced, after a good deal of preliminary terminology, which deals essentially with geometrical ideas that remain unaltered under 'deformations'. For a normed space the fundamental results are the continuity criterion and a theorem of F. Riesz characterizing finite dimensional spaces, the latter being vital in spectral theory. The next two chapters deal with Hilbert spaces and with spaces of continuous functions, including the Stone–Weierstrass approximation theorem and some applications. It is at this stage that the reader comes to a presentation of the differential calculus. The theorems are familiar to all mathematicians, but are presented, against the background of the previously developed ideas, in a way which will be new to many readers. This formulation, involving greater abstraction and more mental effort, is considered worth while because it prepares the reader for the ideas of the calculus on a differentiable manifold. There follows a chapter on plane topology (the method of Eilenberg). Then analytic functions with an appendix on applications. A chapter on existence theorems presents results in the theory of differential equations, these results being linked with the notion of completeness. Finally, there is a chapter on spectral analysis, a subject chosen because it draws on all the material presented earlier in the book and leads to one of the main branches of functional analysis.

Apart from its actual mathematical content, the book is especially important because of the impact it will have on mathematical analysts, as to what should be taught to students of pure mathematics and how it should be presented. Prof. Dieudonné is particularly outspoken about several matters. For example, there is no mention of the theory of the 'Riemann integral', and the author states: "It may well be suspected that, had it not been for its prestigious name, this would have been dropped long ago, for (with due reverence to Riemann's genius) it is certainly quite clear to any working mathematician that nowadays such a 'theory' has at best the importance of a mildly interesting exercise in the general theory of measure and integration. Only the stubborn conservatism of academic tradition could freeze it into a regular part of the curriculum, long after it has outlived its historical importance". For another example, it may be noted that in the chapter on analytic functions the common idea of a 'multiple-valued' function is not introduced, and reasons are given why this idea is limiting and undesirable.

It is important that the reader should understand the aim of this book as well as its contents. For it is a book that lives: it has orientation as well as content and the orientation is justified. At the present time, when there is considerable heart-searching as to what should constitute a curriculum in pure mathematics and how it should be presented, it is particularly fitting that this book should appear, for it may help to clear the way in a somewhat twisted jungle of ideas and prejudices. It may be helpful to quote a part of Prof. Dieudonné's introduction:

"My opinion is that the graduate student of to-day must, as soon as possible, get a thorough training in this abstract and axiomatic way of thinking, if he is ever to understand what is currently going on in mathematical research. This volume aims to help the student to build up this 'intuition of the abstract' which is so essential in the mind of a modern mathematician.

"It is clear that students must have a good working knowledge of classical analysis before approaching this course."

L. S. Goddard

AGE AND KINSHIP IN AUSTRALIA

Classification of Kin, Age Structure, and Marriage amongst the Groote Eylandt Aborigines

A Study in Method and a Theory of Australian Kinship. By Frederick G. G. Rose. (Deutsche Akademie der Wissenschaften zu Berlin. Völkerkundliche Forschungen, Band 3.) Pp. xii+572. (397 Abbildungen.) (Berlin: Akademie-Verlag; London: Pergamon Press, 1960.) 100*s*. net.

This book concentrates on two highly important matters in the analysis of descent systems: the significance of relative age, and the factual documentation of the constitution and working of particular systems. The society dealt with is the

Ingura or Wanindijaugwa of Groote Eylandt, off the northern coast of Australia. The account is based on about four months research in 1941, at which time the population numbered between 300 and 350 individuals. They were organized into eleven localized totemic patriclans, these being grouped into unnamed exogamous moieties.

The major argument is that 'gerontocracy' (the practice of marrying young girls to considerably older men) leads to the abandonment of the general Australian rule of bilateral cross-cousin marriage, and thence to the adoption of the matrilateral rule. The author tries to demonstrate this by assessing the ages of individuals and relating the resultant age-distribution by sex to possible rules of marriage. He shows, for example, that there is an average difference of 15 years between 'full cross-cousins', the patrilateral being the older. It is therefore argued that under gerontocracy this relative could not be married, so that neither patrilateral nor bilateral cross-cousin marriage is possible in this society. But this conclusion relates only to first cousins, whereas typically in a lineal descent system it is a more extensive class of relative, not defined primarily in genealogical terms, which is covered by the marriage regulations. If, then, we consider the ages of all members of the indigenous categories concerned, we have reason to expect a different situation; and in fact the case of individual No. 6, selected solely according to sex (male) and age (23), gives the following results: seven female patrilateral cross-cousins, average age 37; fifteen matrilateral, average age 36. If this case is typical, as on general grounds we may presume it to be, it shows that it is simply the author's European prejudice towards a genealogical definition of status which frames his argument and lends it apparent support.

Moreover, the account of the system is defective. As might be expected with such a short investigation, and an imperfect knowledge of the language, the ethnography is slight and unsatisfactory. The relationship terms seem, on the whole, to indicate an eight-section system, but the genealogical specifications reported are insufficient to establish the precise features of the actual system. Certain positions are not covered by the specifications, and there is an untypical distinction of patrilateral from matrilateral cross-cousin. It is clear, however, that the form of social classification is, contrary to the author's odd assertion, neither 'Kariera' nor 'Karadjeri' (he thinks the 'Aranda' type of system never existed). Also, the normal marriage is said to be with the mother's mother's brother's daughter's daughter who is a 'distant' mother's brother's daughter; but this cannot be reconciled with the system apparently framed by the terminology. Moreover, the abundant tabular data, occupying 220 pages of the book, are no substitute for genealogies, details of group-composition, and territorial distribution, let alone the indigenous ideology, and they cannot yield the evidence which is necessary to structural investigations of the kinds suggested by the author's argument.

In view of the interest of the issues he deals with, Dr. Rose's study is particularly exasperating. His record of ages is precisely the kind of exact evidence which is essential to the comprehension of Australian societies, and he is right to deplore its absence from other accounts; but without a comprehensive structural examination, conducted primarily in terms of the categories employed by the people themselves, this record is of depressingly little sociological use. It is possible, however, that it may serve certain purposes; and the work also includes percipient and forceful observations on a variety of other topics in the study of aboriginal Australian society (see, for example, his criticisms of Radcliffe-Brown's statements about the Kariera). Inadequate though it must be judged, and fallacious as its central argument almost certainly is, Dr. Rose's monograph is a singular (if eccentric) contribution to the field and deserves a place in any library of social anthropology.

R. NEEDHAM

SOME ASPECTS OF HÆMATOLOGY

Lectures on Haematology
Edited by F. G. J. Hayhoe. Pp. viii+247+24 plates. (Cambridge: At the University Press, 1960.) 60*s*. net.

THE chapters in this book represent a course of thirteen lectures delivered to an audience of post-graduate clinicians and medical research workers. Most of the information has been published elsewhere, but this is in no sense a text-book of hæmatology. Each chapter is a survey, by an expert, of some rapidly expanding or controversial topic in the field. The accounts are of necessity brief, and only some of the most recent work is covered in detail. A good bibliography at the end of each chapter brings in the earlier literature.

The first five chapters deal with abnormalities in production and reactions of erythrocytes. J. V. Dacie writes on acquired hæmolytic anæmia, W. M. Davidson on iron metabolism and iron-deficiency anæmia and R. H. Girdwood on the pathogenesis and differential diagnosis of megaloblastic anæmias. The hazards of blood transfusion are discussed by G. T. Discombe, and J. C. White gives an account of hæmoglobin variants.

Although these chapters are directed primarily to the audience concerned they will provide useful information for a much wider circle of readers. Anyone concerned with the preparation or administration of blood for transfusion would profit from reading this entertainingly written and eminently practical account of the hazards involved. Especially since, as the author mentions, there is at present no comprehensive review of the subject in the English language.

Chapters 6–10 deal with various aspects of leukæmia. F. G. J. Hayhoe, who is also the editor of the book, considers the clinical and cytological recognition and differentation of the leukæmias. G. Wetherley-Mein writes on the myeloproliferative syndromes, and R. Doll on the incidence of leukæmia and the significance of environmental factors in its causation. The chemotherapy of leukæmia is discussed, as a practical problem, by D. A. G. Galton, and H. E. M. Kay writes on the potentialities of marrow transplantation in the treatment of leukæmia.

The last three chapters deal with abnormalities in hæmostasis. R. M. Hardisty discusses the contribution of blood and blood vessels in the hæmostatic process. Congenital and acquired disorders of coagulation are reviewed by G. I. C. Ingram and the pathogenesis of purpura by J. F. Ackroyd.

A great merit of the book is that it is easy to read, for in most cases the lively and personal approach of a lecture has survived in the printed account.

M. E. NUTT

Fast-Neutron Spectroscopy

By B. V. Rybakov and V. A. Sidorov. Edited by N. A. Vlasov. Translated from the Russian. (Supplement No. 6 of the *Soviet Journal of Atomic Energy*, 1958.) Pp. ix+121. (New York: Consultants Bureau, Inc.; London: Chapman and Hall, Ltd., 1960.) 17.50 dollars.

DURING the past few years neutron spectroscopy in the energy-range of 0·3–30 MeV. has made enormous progress, and in this book we have a review of the field up to 1958. Since this is a Russian work the English translation has not appeared until recently and so is already partly out of date. Nevertheless, it has especial interest because of the coverage it gives of fast-neutron spectroscopy in the U.S.S.R.

The most powerful technique which has been introduced into this field recently is the time-of-flight method. Development of fast pulsed sources and fast detectors and electronic equipment has given this method its present overall usefulness. Approximately two-thirds of the book is given to a valuable discussion of these techniques. The authors have been working with the cyclotron at the U.S.S.R. Institute of Atomic Energy and have considerable experience of time-of-flight work. Their description of this field covers their own equipment and experiments, in addition to others.

There are as well chapters covering methods which have a more specialized application. Such methods as proton-recoil, helium-3 spectrometer and scintillation counter techniques are covered adequately.

This review is intended for people already working in this field or about to do so; a graduate background of physics is assumed. The style is fair, some clarity and emphasis being lost in translation. However, the actual technical translation errors do not seem to be many. From the aesthetic point of view the cover design is depressing and the print is tiring. Probably most scientists would find it a relatively expensive book.

In conclusion, this book could be recommended as good reading, but scarcely worth buying for an individual scientist's library. P. A. Egelstaff

Atomic and Nuclear Physics

By Prof. Robert S. Shankland. Second edition. Pp. xv+665. (New York and London: The Macmillan Company, New York, 1960.) 49*s*.

THIS is the second edition of Prof. Shankland's undergraduate text-book. It is cast in the form of fairly brief lecture notes with a minimum of mathematics and perhaps one typical experiment for each topic. The range of physics covered is very broad and admirably up to date. Electron physics, molecular physics, solid state, nuclear and high-energy physics and reactor physics are all included. Although the underlying unity of microscopic physics is implied by including them all in one book, the clear demonstration of this is seriously impeded by the rather traditional and individual presentation of each field. That is, the partial emphasis on historical development is not sufficient to make this aspect very clear, but makes it difficult to see the elegant unity which has been achieved with the help of quantum mechanics.

This is a criticism of the overall organization of the book. The individual topics are treated clearly and simply and in each case carefully selected references to the original literature can lead the student to the details of the work. The experiments described for illustrative purposes are generally very modern ones. About 350 problems are collected at the end of the book.

This text is on the whole a very good example of its kind, especially if accompanied closely by lectures in experimental physics and quantum mechanics. E. B. Paul

Mammals of Northern Rhodesia

A Revised Check List with Keys, Notes on Distribution, Range Maps, and Summaries of Breeding and Ecological Data. By W. F. H Ansell. Pp. xxxi+180+7 plates. (Lusaka: Government Printer, 1960.) 12*s*. 6*d*.

FAR more than a checklist, this is really an admirably produced pocket guide, into which the maximum amount of information has been compressed without losing simplicity of arrangement and ease of reference. With identification keys, notes on habits, habitats and distribution, as well as seven useful colour plates by P. Dandelot, it is a model of its kind. Both the introduction, with its stress on the need for further collecting before the numbers and range of the mammals are irrevocably reduced by human activity, and the discussion of problems of habitat, ecology and distribution, are well calculated to stimulate or arouse interest in these aspects of the subject. The book will also be of much practical value to the professional working on the mammals of Central Africa. Sections listing rejected species, and species likely to be included as a result of further investigation, help to complete the work, and separate indexes of English, scientific and vernacular names help easy reference.

The only criticism relates to the unfortunate printing of the seventeen full-page distribution maps sideways without a corresponding adjustment of the captions. In two cases, zebra and lechwe, where cross-hatching indicates the distribution of local races, the result is the opposite of what was obviously intended. R. W. Hayman

Remington's Practice of Pharmacy

Edited by Eric W. Martin, E. Fullerton Cook, E. Emerson Leuallen, Arthur Osol, Linwood F. Tice and Clarence T. Van Meter. Twelfth edition. Pp. xii+1866. (Easton, Penn.: Mack Publishing Company, 1961.) n.p.

DR. FULLERTON COOK has now played an important part in producing successive editions of this large standard work for more than 60 years. It deals with the whole of American pharmacy from history and ethics to poison control. It contains useful information about all the sciences which a pharmacist must understand, and there are more than 600 pages devoted to the properties of the drugs used in medicine. These drugs are, for the first time, classified pharmacologically, in recognition of the fact that pharmacists are now consulted about the actions of drugs, but a summary of the chemical classification is also included. Among the subjects dealt with in the various parts of the book are statistics, rheology, chromatography, sterilization, methods of manufacture, sustained action medication, aerosols, parasiticides, pesticides, diagnostic drugs, allergenic extracts, radiopharmacy, analytical pharmacy, immunization and many other things The information on all these topics is full and up to date. The total

number of pages has increased by 160 since the last edition was published five years ago. The six editors, 56 assistant editors and more than 160 contributors are to be congratulated on keeping this reference book up to date. J. H. GADDUM

Sofiano (T.A.) English–Russian Geological Dictionary

Edited by I. D. Borneman *et al.* State Publ. Office Techn. Theor. Literature, Moscow, 1957. Pp. 525. Price 17r. 90k.

Sofiano (T.A.) Russian–English Geological Dictionary

Edited by A. P. Lebedev and V. E. Khain. Central Editorial Board, Foreign Language Scient. Techn. Dictionaries, Fizmatgiz, Moscow, 1960. Pp. 559. Price 18r. 60k. (old roubles), 1r. 86k. (new roubles).

THESE two dictionaries, well printed and bound, and of a convenient size (21 × 14 × 3 cm.), contain some 35,000 terms each. The majority of these terms consist of one word, but quite a number of them are adjectival terms, such as, for example, 'deltaic fan', or even more complex phrases like 'planar flow structure'. Besides purely geological, petrological and mineralogical terms there is a number of mining, civil engineering and allied physical, chemical and biological terms. Some adjectival terms are placed under their main term. Thus the term 'sand' is followed by 'arkose sand', 'coral sand', etc. On the whole the compiler of these two dictionaries, Madame T. A. Sofiano, has been rather generous in her collection of terms, which is all to the good. As with other dictionaries, there are occasional mistranslations. The English–Russian dictionary has also a list of English abbreviations, which is not very satisfactory, but there is no list of Russian abbreviations. In spite of these minor defects these two dictionaries represent extremely valuable auxiliary reference books for geologists. S. I. TOMKEIEFF

Linear Programming

Methods and Applications. By Saul I. Gass. Pp. xii + 223. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 50*s*. 6*d*.

Methods of Feasible Directions

A Study in Linear and Non-Linear Programming. By Dr. G. Zoutendijk. Pp. vi + 126. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 30*s*.

THE first book is a formal mathematical text on linear programming and is to be distinguished from the many general accounts on the subject that have appeared. It contains much information that could in the past be obtained only from original papers. Although writing for those with mathematical leanings, the author has a constant eye on the practical applications of the methods he describes. The book is divided into three parts. The first contains a general introduction and some necessary mathematical background, the second describes the theoretical and computational methods available for use, and the third discusses applications. The book has already become well known in the short period that has elapsed since its publication and is to be highly recommended.

The second book has the sub-title *A Study in Linear and Non-Linear Programming*, and is in the nature of a research monograph. Its appreciation requires a knowledge of present-day linear programming principles and techniques such as might be obtained from the foregoing book. The first half is concerned with linear programming, and in particular with a series of improvements to the product-form algorithm. In favourable circumstances these enable substantial economies to be made in the amount of computation required.

The second half of the book is concerned with methods of solving the general convex programming problem. In all these methods one starts with a feasible solution, determines a feasible direction of advance which will increase the value of the solution, and takes a step of appropriate length in that direction. There are various methods of determining the feasible direction of advance and the length of the step to be taken. Some of these described reduce in the case of linear or quadratic problems to methods which are already well known.

M. V. WILKES

Digital Computers and Nuclear Reactor Calculations

By Ward C. Sangren. Pp. xi + 208. (New York and London: John Wiley and Sons, Inc., 1960.) 8.50 dollars; 68*s*.

PROSPECTIVE readers might not gather from the title of this book that the first half contains a valuable general introduction to digital computers written by a very experienced user. This is a pity, since others than nuclear physicists and engineers might profit from this part of the book. The chapter on programming in particular has an authentic ring about it. The chapter on numerical analysis is perhaps less successful being rather too compressed for the novice and neither sufficiently comprehensive nor sufficiently critical to appeal to the expert.

The last four chapters are concerned with the solution of technical problems arising in reactor design and in other branches of nuclear engineering; their scope is indicated by their titles: "A Code for Fission-Product Poisoning", "Diffusion and Age-Diffusion Calculations", "Transport Equation—Monte Carlo", "Additional Reactor Calculations". There is a useful selected bibliography. The author remarks that the problems he describes are good examples of problems in mathematical physics that can be 'solved' sensibly with digital computers: he considers, therefore, that they should interest not only reactor specialists but also applied mathematicians, physicists, and persons professionally interested in computer applications.

M. V. WILKES

Birds of Eastern and North Eastern Africa

By C. W. Mackworth-Praed and C. H. B. Grant. Second edition. (African Handbook of Birds, Series 1, Volume 2.) Pp. viii + 1113 + plates 54–96 + photographs 7–19. (London: Longmans Green and Co., Ltd., 1960.) 50*s*. net.

THIS is the indispensable work of reference for all who are interested in the birds of East Africa, and it is not surprising that the demand for copies has called for a second edition. The two volumes were originally published in 1952 and 1955; a second edition of Volume 1 has already appeared and the revision is thus now complete. The work having been reproduced by photo-lithography, the changes are necessarily limited to additional matter inserted in spaces which had been left for the purpose and on some extra pages at the end. A supplementary index to the addenda and corrigenda is conveniently provided. LANDSBOROUGH THOMSON

THE MAXWELL COLOUR CENTENARY

AFTER all the recent questioning caused by Dr. Edwin Land with his demonstrations of two-colour projection, the principles of three-colour reproduction came into their own again at the Maxwell Colour Centenary Conference held at the Imperial College of Science and Technology, London, during May 16–18, under the joint auspices of the Colour Group (Great Britain) and the Inter-Society Color Council (U.S.A.), and attended by about 220 members from twelve countries. The particular centenary to be celebrated was the lecture delivered by Maxwell at the Royal Institution on May 17, 1861, when he gave the first public demonstration of three-colour reproduction by projecting images in red, green and blue light in superposition on a screen.

The Conference was fortunate to be able to arrange a centenary discourse at the Royal Institution itself exactly a hundred years after Maxwell's lecture. The subject of the discourse was "A Hundred Years of Colour Photography", and it was delivered by Dr. D. A. Spencer (Kodak, Ltd., Harrow). One of the most interesting items in the lecture was the report of a piece of detective work by Dr. R. M. Evans, of the Eastman Kodak Company in Rochester, U.S.A., leading to the explanation of how Maxwell had succeeded in obtaining an acceptable colour reproduction at a time when photographic emulsions were sensitive only to wave-lengths extending from the blue part of the spectrum downwards into the ultra-violet. Evans provided convincing evidence to show that, in securing his red separation negative, Maxwell had unwittingly taken advantage of the ultra-violet transmission common to many red dyes and filters. Further, the green filter must have had just sufficient transmission at the long-wave end of the blue part of the spectrum to enable Maxwell to secure a distinctive exposure for the green separation negative. By these fortuitous circumstances Maxwell was thus able to produce a three-colour photograph some twenty years before photographic emulsions sensitive to the green and red had been produced.

The theme of the Conference itself progressed from a study of the facts of three-colour mixture and their biological interpretation to their application in photography, television and printing, with a final day devoted to a discussion of colour appearance. The programme was not overburdened with papers, three introductory talks in each half-day being the normal arrangement, followed by informal discussion. Not all the speakers kept to their allotted 20 min., but at most of the sessions there was a satisfactory division between prepared and extemporary contributions.

The paper by Dr. D. B. Judd (National Bureau of Standards, Washington) on the opening morning had a detective flavour similar to that of Dr. Evans's study, since he had made a fresh analysis of the colour mixture curves obtained by Maxwell and was able to show that they were in closer accord with the Commission International d'Eclairage standard curves than had generally been realized. Maxwell's theoretical ideas, too, had anticipated many of the basic concepts of 'modern' colorimetry, and Dr. B. H. Crawford (National Physical Laboratory, Teddington) reported some new results from the National Physical Laboratory on large-field colour matching which had been inspired by Maxwell's original experiments. In Maxwell's method, colour mixture curves can be derived by mixing two fixed wave-lengths with a third, variable, wave-length so as to match a constant white, thus providing fixed adaptation conditions for all the matches. Crawford found a significant difference between the mixture data calculated from this type of observation compared with those derived directly from more orthodox experiments in which three fixed red, green and blue wave-lengths are mixed to give a match of each wave-length in the spectrum in turn after suitable desaturation. This disagreement may provide a possible clue to the small, but significant, discrepancies in the field tests made on the large-field colour mixture functions prepared by the Colorimetry Committee of the Commission Internationale de l'Eclairage from the Stiles–Burch–Speranskaya data. However, if Crawford's results are confirmed, they imply in effect a failure of additivity in large-field colorimetry, a possibility that has already been the subject of some discussion and which will no doubt be investigated more fully in the near future.

Perhaps the most original contribution to the meeting came from Dr. W. S. Stiles (National Physical Laboratory, Teddington) and Dr. G. W. Wyszecki (National Research Council, Ottawa), when they considered the spectrum as divided into M small wave-length intervals. They then showed that there was a statistical limit to the number of different selections of these M elements which would combine together to reproduce a given colour, ranging from a minimum number of combinations for colours in the spectrum to a maximum for a white surface. Lights of different spectral composition which have the same colour are known as metamers, and Stiles and Wyszecki showed that 'counting metamers' might prove of value, for example, in assessing the colour-rendering properties of two illuminants or comparing the merits of two sets of colour-matching functions.

In the biological session, we were reminded once again, by Dr. W. A. H. Rushton (Trinity College, Cambridge), Prof. Yves Le Grand (Natural History Museum, Paris) and Prof. R. W. Ditchburn (University of Reading), of the complexities of the visual process. However, the ophthalmoscopic arrangement used both by Rushton and by Weale for measurements of the reflectivity of the fundus has led to some surprisingly direct information about the density changes in the visual pigments during bleaching and regeneration, and about their spectral sensitivity. Rushton, indeed, is obtaining gratifying confirmation of the type of colour-mixture curves associated with the classical three-components theory of colour vision, and as derived, for example, by Pitt from work on the dichromatic form of colour defect.

Nevertheless, it is clear from the work on eye movements and on the stabilized retinal image at the University of Reading under Prof. Ditchburn, that the scanning of the retinal image plays a vital part in colour discrimination. Indeed, it seems almost certain that time-coding of the neural in-

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formation must occur at some stage in its transmission from retina to visual cortex, and Ditchburn suggested that the colours we see may be determined very largely by edge effects occurring at the boundaries separating one area of colour from another in the retinal image.

However that may be, we can agree with Le Grand that the one certainty that emerges at the moment is the complexity of the visual processes and their interactions at all stages. Yet to a question about the possibility of the retinal receptors behaving like wave-guides and of their colour sensitivity being determined by their physical structure, Rushton was prepared categorically to deny this on the evidence of the colour of light scattered sideways into the receptors from other illuminated parts of the interior of the eyeball.

May 17 was devoted to discussions of problems of colour reproduction, and it was fascinating to compare and contrast the differing situation facing photographers, television engineers and printers. Colour photography depends on subtractive colour mixtures using yellow, magenta and cyan dyes; but the dyes that have to be used possess unwanted and overlapping absorptions. Hence no simple relation exists between exposure and density of colour, so that some colour distortion is inevitable. Defects of colour quality in one direction have therefore to be offset by deliberate distortions in the opposite direction; but the enormous popularity of colour photography is a tribute to the successful compromise which has been achieved. Here, as Mr. R. G. Horner (Ilford, Ltd., Brentwood) acknowledged, the eye is very accommodating and tries its best to see things as the brain thinks they should be seen.

Colour photographic prints present greater problems than colour transparencies, and Dr. R. W. G. Hunt (Kodak, Ltd., Harrow) explained how manufacturers had been able to take advantage of the fact that the light from the great majority of colour pictures integrates to grey. Photo-electric methods of controlling the colour balance can therefore be used, although the 'integrating to grey' method is sometimes criticized because of the colour distortion that occurs with special subjects. Mr. L. C. Jesty (Sylvania–Thom Colour Television Laboratories, Ltd.) jokingly congratulated Dr. Hunt because he had described this limitation as 'colour failure', whereas to the disappointed customer it is commonly described as 'subject failure'.

In many ways the situation in colour television is more straightforward. The picture is reproduced by additive colour mixture, the laws and data of trichromatic colorimetry can be fed directly into the system, and transformations of the colour information to a form best suited for transmission and reception can be achieved by the electronics engineer in a box which he labels 'matrix'. All this was admirably surveyed by Mr. J. W. Wentworth (Radio Corporation of America); but the television engineer is still left with the major problem of simplifying and reducing the cost of the colour receiver. The most expensive item, of course, is the colour tube which, in its more common form, comprises a three-electrode gun, a shadow mask and a phosphor mosaic. It would be ironic if someone invented a cheaper receiver based on subtractive colour mixture, and so deprived television of some of the theoretical simplicity of an additive system.

Colour printing is in a different position still, since pigment mixture, as Dr. D. R. Duncan (Paint Research Station, Teddington) emphasized, involves both absorption and scattering and is therefore neither purely subtractive nor purely additive. Hence, as was made clear both by Mr. D. C. Gresham (McCorquodale, Ltd., London) and Mr. W. L. Rhodes (Rochester Institute of Technology) colour printing is very much an empirical process that does not lend itself to any neat theoretical or mathematical solution. Moreover, for various technical reasons, including the need to sharpen the fine detail, it is often necessary to include a black printer. Colour printing then becomes a four- instead of a three-colour process. The skill of the craftsman ensures that excellent colour prints can be made, but the demand for mass printing makes automatic or semi-automatic colour correction a very attractive proposition, and Mr. V. C. Hall (Time, Inc., Springdale) described how this can be done with scanning machines and scanner computers. However, so far, only some 10 per cent of colour prints are made in this way.

The subject for the third day of the Conference could be summed up as 'colour as we see it'. If any physicists present were still under the delusion that the physical stimulus was all that mattered they were rudely disabused by the lively discussion that developed on colour appearance and how it could be studied. Miss Dorothy Nickerson (U.S. Department of Agriculture, Washington) was concerned with the possibility of exploiting the Munsell variables of hue, value and chroma as the co-ordinates in a system of appearance specification, with the colour-rendering properties of light sources particularly in mind. Dr. Crawford also discussed the problem of colour rendering, and he showed how the remembered appearance of known objects, or memory assessment, had proved a valid method of study. Prof. H. Helson (University of Texas) described some experiments in which the appearance of coloured shadows was in fact expressed in terms of the Munsell colour chips from measurements made by a binocular matching technique. Mr. W. C. Granville (Granville Color Service, Libertyville) demonstrated the critical selection of colours which we make in designing a colour scheme, especially in schemes involving near-neutral colours. He considered that in the colour atlases in common use many more samples of low saturation were required in the neighbourhood of the neutral axis.

Mr. M. H. Wilson (Goethean Science Foundation, Stowbridge) took us back to Goethe and treated us to some very effective demonstrations of colour as light and colour as darkness. His description of additive mixture as the mixing of coloured lights and subtractive mixture as the mixing of coloured darknesses gave the audience just the mental shock necessary to keep a conference alive. No doubt to the orthodox physicist such phrases sound like nonsense, but the subject of colour is one where the physicist soon has to leave much of his orthodoxy behind him. Certainly one of the most valuable aspects of the meeting was its success in bringing colour experts of very different backgrounds and experience together into intimate and informal discussion.

For the Colour Group it was an auspicious start to its new existence as an independent body, and Dr. Hunt, the present chairman of the Group, paid a fitting tribute to the Institute of Physics and the Physical Society both for help in organizing the meeting and for the manner in which the Group was enabled to separate from the parent body and launch into an orbit of its own.

W. D. Wright

STORAGE AND RETRIEVAL OF BIOLOGICAL INFORMATION*

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MANY problems of storage and retrieval of biological information are common to those of scientific information in general, and to give perspective in a connotation where 'information' is usually thought of as synonymous with 'the literature' certain peculiarities of scientific literature must be noted before considering some characteristic features of biological information.

Much of the best scientific literature is ephemeral. Its classics are seldom read. Scientific work is firmly based on the findings of other scientists, and is brought to a right conclusion only when the results are made generally available. In other words, there must be communication, and the modern tempo requires this communication to be rapid. Further, while much literature in the ordinary sense is national, science, and hence scientific literature, are international, and the prestige value of science coupled with the growth of nationalism during recent years has increased the scatter of the languages in which scientific results are reported; but this has been counteracted by the desire of authors to be widely read. The amount written or summarized in English continues to increase.

For biology, as for science in general, the size of the literature and the current output are not known with any certainty. In February this year *Biological Abstracts*, begun in 1926, published its millionth abstract. This comprehensive abstracting journal for all branches of biology except clinical medicine is at present increasing its coverage. This year 87,000 abstracts are promised, and by next a rate of 100,000 abstracts a year is to be attained. But such a coverage would be incomplete, for a recent World Health Organization survey[1] has estimated that the annual output for biology and medicine approaches 400,000 articles and more medical and veterinary literature than non-medical biologists like to think has biological interest (and there are 4,000–5,000 medical periodicals containing about 230,000 papers each year[2]), while biologically relevant data are reported in the fields not only of biochemistry and biophysics but also of chemistry and physics, statistics and semantics, anthropology, psychology, and so on. Admittedly the biological data are few in some of these studies; but probably more fruitful connexions could be made if some specialists were less specialized.

The extent of the literature on some specific biological topics is more accurately known. For example, in 1953, for plant protection in the wide sense, the latest (1960) issue of the annual *Bibliographie der Pflanzenschutzliteratur* records 15,000 items obtained by scanning some 3,000 periodicals, and for fungus diseases of higher animals and man a census of the publications for 1957 has so far yielded 1,105 items. Further, as an example of an estimated total, there are not less than a quarter of a million books and papers on fungi.

As for science in general, the bulk of biological literature is stored as journal articles, and here a rather curious state of affairs may be noted. Although any investigation is only completed when the results are communicated and although State (or other) funds are usually available, if on a modest scale, for scientific investigation, very little money indeed is made available for the costs of publication, as Mellanby[3] has pointed out. Biologists have to unite in co-operatives (or 'publishing societies') and arrange for the publication of their own work. The State recognizes the value of such societies by allowing remission of income tax on an individual's subscriptions to approved societies, by permitting more altruistic members to covenant their subscriptions, and by an annual grant-in-aid of scientific publications (administered by the Royal Society) to enable societies to publish more without going bankrupt. There is a feeling that biological societies have not always received a fair share of this grant-in-aid. For 1959–60, however, biological societies received half the grant distributed[4], but as more than half remained undistributed there seems scope for more importunity by the smaller societies, particularly those with a taxonomic bias, all of which have at times had to restrict their activities owing to printing and publishing costs.

This traditional method of publication has disadvantages. It is wasteful, for the economics of many periodicals depend on the subscribers, both individual and institutional, receiving much material they would never otherwise have bought, thus preventing its availability to others who need it. This has long been realized, and a recent Unesco report and bibliography by Phelps and Herling[5] lists more than a hundred papers and reports on alternatives to the scientific periodical. But nothing is done. The traditional method still works. The position is not yet intolerable.

Biology differs from most other branches of science in that an important part of its information is stored not as literature but as specimens. Even the simplest objects of a biologist's study are complex, and while great progress has been made in describing living things in words, by illustrations, and by musical notation there are frequent uncertainties of identification or problems of affinity which can be resolved only by the comparison of specimens or tape recordings: hence the many collections of specimens, from those of the large national herbaria and zoological and geological museums to the personal collections of individuals. The specimens need not, however, be dead or fossilized. There are national parks and nature reserves, zoological and botanical gardens with their diverse regional and exotic contents, culture collections of micro-organisms of all groups, and one cannot exclude collections of cultivated plants and domesticated animals which shade off into the accumulations of fanciers, whether of tropical fish or alpine plants. All in their different ways contribute to the store of basic biological information.

The retrieval of specimens, which is not always easy (for their cataloguing often leaves much to be desired), is a topic which cannot be given detailed consideration here. It must be sufficient to indicate present trends by recalling that since 1952 the *Index*

* Substance of a lecture to the London Branch of the Institute of Biology, April 27.

Herbariorum[6] (already in its fourth edition) has provided a handy directory to the herbaria of the world (and this work is in the course of supplementation by a list of collectors represented in public herbaria) and that the Unesco-sponsored International Federation of Culture Collections of Microorganism at Lausanne, founded in 1947, attempts to locate for inquirers any cultures wherever they may be available.

The storage of biological literature should be integrated with the retrieval pattern, and so it is convenient to consider the rationale of storage and the retrieval pattern together.

The most frequently employed retrieval method can only be noted. All 'reading habits of scientists surveys' are in agreement that the first reaction of any scientist needing information is to ask a colleague who it is thought may know the answer. Only when this fails are other avenues explored.

Retrieval can be divided, if somewhat arbitrarily, into retrieval of current literature and retrieval from literature of the past. Retrieval from current literature begins with the arrival of members' copies of society journals. This is supplemented by visits to the departmental library, reprints from others working in the same field, abstract journals, etc. From such sources every research worker notes papers of current interest which he stores in his head or in a more or less convenient and more or less haphazard manner, depending on his temperament and the quality of his secretarial support. For some, noting information becomes obsessional. For most it is a drudgery to evade.

It does seem that a primary requirement for rapid retrieval is an efficient 'contents of current journals' service. *Current Contents*, issued by the Institute for Scientific Information, Philadelphia, with the collaboration of journal proprietors, offers a weekly list of the contents pages of more than six hundred journals in the "chemical, pharmaco-medical, and life sciences". More analytical, but not necessarily more useful, monthly surveys are provided by the well-subsidized and therefore relatively inexpensive *Bibliography of Agriculture* (based at the Library of the U.S. Department of Agriculture, Washington) and the *Index Medicus* (National Library of Medicine, Washington). At the beginning of 1960 the last of these was re-organized and its production mechanized. Each of these periodicals lists 100,000 or more titles a year and each is integrated with a photocopying service. Descriptive zoology is covered in a more leisurely manner by the *Zoological Record*, veterinary science by the *Index Veterinarius*, and more in this way might be done. At the moment suggestions for an *Index Biologicus* are being canvassed.

The next step is provided by the diverse abstracting organizations which scan the literature independently, usually with particular audiences in mind. Much could be said about these services. One generalization is that any item abstracted is likely to be abstracted more than once. The World Health Organization survey already mentioned estimated that 246 journals from 10 countries publish more than one and three-quarter million abstracts a year, that is, more than four times the total output of medical and biological articles.

Retrieval from the past is fraught with difficulties. Memories are short, many special libraries are young, and the searching of annual and cumulative indexes is tedious and very uncertain of results. (One inevitable imperfection of indexing is that indexers can never be sure what posterity will find relevant.)

Biology has one major advantage over other branches of science in having a built-in, internationally used, basic retrieval code (which also reflects a more or less internationally acceptable classification) in the scientific names applied to living things, especially at the generic and specific levels. The truly international character of the binomial names of species is not always fully appreciated. Such Latin names are frequently the only means by which a Western biologist can ascertain the subject of writings in Russian, Japanese, and Chinese. When this is realized, the importance of using names in line with what may sometimes seem to be the arbitrary requirements of the three *International Codes of Nomenclature* (those for zoology, botany and bacteriology) should be apparent.

Scientific names are also the basic grid references by which one experimental biologist is able to relate his observations to those of others. There are, of course, pitfalls. Changes in names necessitated by taxonomic studies tend to give taxonomists an unjust reputation for irresponsibility. Many taxonomists are well aware of the undesirability of disturbing traditional nomenclature. Unfortunately the cataloguing of organisms is far from complete, and many necessary taxonomic changes (and hence changes in names) will have to be made.

One thing that taxonomists are not required to do under the *Codes* is to register their nomenclatural innovations. It is left to others to find the published announcements, and that may take decades. Many names are thus duplicated, and unnecessary confusion results. Even the latest supplement (that for 1950–55) to the *Index Kewensis* (which since 1895 has been compiling the names of the genera and species of flowering plants) has a spread of more than a hundred years. (The average lag in retrieval by current content and abstract journals of, say, 75 per cent of the literature noticed is probably about 9–12 months, a period which approximates to the interval between the submission of a paper to a journal and its subsequent publication.)

There is another outstanding need. While there are lists of varying length and varying degrees of incompleteness of the genera and species of most of the main groups, there are very few complete lists, and no general compilation. A list of the genera proposed for all organisms would be a most valuable tool not only for biologists but also for librarians, information officers, and so on. By consulting such a list, information could be correctly classified for filing, the direction in which to look for further information indicated, and the duplication of names would be less likely. The systematic position of each name could be briefly coded (on the lines suggested by Mullins and Nickerson[7]) and the work, which should be internationally sponsored, need not be larger than one of the sectional volumes of the *London Telephone Directory*—and might be of similar quality and format, for such a compilation would need to be under constant revision, and it would be rational when the latest arrived to discard the previous edition.

This raises the question of the length of life of biological literature. The average life of a physics paper is said to be 2–3 years. Biological literature, much of it still being descriptive, is rather longer-lived although some useful material scarcely survives birth.

During the past two years more than a hundred papers have been published to show that 1 gm. daily of griseofulvin taken orally effectively controls infection of man by diverse dermatophytes in all five continents. Many of these papers were necessary. Some are of high quality and beautifully printed on good paper. This should not mislead. They are mostly already as dead as last night's evening newspaper. They will not be consulted again, but they will survive for long in indexes and bibliographies and so tend to obscure still useful data on a notable antibiotic.

At the other extreme taxonomic biological literature is long-lived. A comprehensive taxonomic paper may have an effective life of anything from fifty to a hundred and fifty or more years and at centres where general taxonomic investigation is undertaken works a century old are in constant use at the bench.

For science in general the small use to which much of the literature is put has been brought recently into prominence by the survey by Urquart and Bunn[8] of the use made of the Science Library serial holdings during 1956. They found that, of the total of 9,120 holdings, 350 titles accounted for 50 per cent of the total use, 1,200 for 80 per cent, and they consider that for perhaps half the present titles the total use is less than 10 times per 10 years. The relevance of this in planning storage is obvious. For some journals one run would be sufficient for the needs of the whole country—provided photocopies or microfilms were loaned—while for bulky, little-used, or rare material microfilms, microcards, or fiche could be used to reduce storage and acquisition costs.

Microtexts (which have recently been most lucidly reviewed by Davison[9]) have many advantages, and to taxonomists it is their retrieval value which appeals so strongly. One of the banes of taxonomic work is one taxonomist copying data from publications not seen from the work of another (who as likely as not copied from a predecessor who, even if he did see the original, probably interpreted what he read; only a very experienced taxonomist can copy what an author actually wrote). A number of useful taxonomic journals and scarce out-of-print books and monographs are already available on cards or fiche. But their production is slow and unplanned, and their cost, the demand being so much below what it might be, relatively high, although cheap compared with originals. In part the small demand is due to prejudice, in part to the lack of a mass-produced reading device, and in large part just ignorance.

One of the chief omissions in this brief survey is a consideration of systems of classification for information storage and for indexing the information stored. Another omission is any mention of the coding and mechanical searching for data. Mechanization begins with the identification of, say, timber-attacking fungi by hand-sorting punched cards with a knitting needle[10], and proceeds via elaborate mechanical sorting of thousands—even tens or hundreds of thousands—of Hollerith or IBM cards to systems of coding on magnetic tape which can be searched at incredible speeds. Some of these methods are of proved value, others are of great potential value. There does, however, seem to be a very real danger of treating comprehensive systems for storage and retrieval as ends in themselves. Much effort can be wasted in coding and storing information for which there proves to be little or no demand. The most urgent present need for biology is for some much simpler aids to information storage and retrieval.

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THE CONCEPT OF KIND OF TEA

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BY tea we mean the 'black tea' usually consumed in Great Britain and elsewhere which is made from the fermented shoots of *Camellia sinensis* (L.) O. Kuntze, and its hybrids. Our remarks do not refer to unfermented 'green tea' or to partially fermented 'oolong tea'.

When one speaks of tea 'quality' two meanings can be conveyed: to the uninitiated the expression suggests the overall value or acceptability of a tea, in the sense of a summation of its desirable attributes; but in the trade, 'quality' is a particular attribute which, by itself, does not determine acceptability.

Attributes of tea recognized by professional tasters have been described in a recent report[1]. Quality and strength are important, and a full understanding of their separate meaning is necessary if tea markets are to be expanded. In our investigations quality has been defined as a dulcet freshness, like that of a recently caught fish, in contrast with one that has been in cold storage. The quality of tea enhances one's appreciation of its odour. A crude analogy is the sugar that imparts a quality to sauce without making it noticeably sweet. A fresh hazel nut (*Corylus* sp.) has the sweetness of quality. Some tasters might disagree with these definitions, but it is usual for their ordination of teas on a 'quality' [*sic*] basis to be very similar. Strength is a bitterness like bitter beer contrasted with mild beer.

Relative intensities of quality and strength, measured by tasters' scores[2], vary from plant to plant. Between populations the two attributes are often positively associated, with a correlation coefficient of the order of 0·6. The morphological feature of pubescence is positively correlated with quality[3]; and under the same conditions a similar

correlation can be expected between pubescence and strength. Whereas pubescence makes roughly equal contributions to these two attributes, other morphological features make unequal contributions: for example, the vascular bundle when defined as the ratio of xylem cells to phloem cells (per unit area of cross-section), makes a positive contribution to quality and a negative contribution to strength, in accordance with the statistically significant ($P < 0.01$) equations:

$$\text{Quality} = -27 + 0{\cdot}06C + 5{\cdot}5H + 0{\cdot}16Pi + 50Vb$$
$$\text{Strength} = 22 + 0{\cdot}11C + 4{\cdot}5H + 0{\cdot}05Pi - 22Vb$$

where C is the crop yield of the bush, H is its pubescence, Pi is phloem index[4], and Vb is vascular bundle. C varied from 86 to 171, H from 1·30 to 3·23, Pi from 20 to 116, and Vb from 0·40 to 0·66. The factors are derived from 10 jat populations. The equations are intended only to indicate the nature of the situation within the limits of *C. sinensis* var. *assamica*. It is not possible to enter into details in the present article. It is, however, important to note that vascular bundle (Vb) differs greatly between biotypes.

It is misleading to say that "The various types of tea in commercial use result mainly from differences in manufacture"[5]. All black tea is not alike, and many differences are due to genetical factors. For example, the kinds of tea called 'Ceylon', 'Darjeeling' and 'Assam', are well known and will serve as examples. In common parlance, these teas 'taste' differently; but much of this effect is due to odours the perception of which is independent of taste buds. Thus it is necessary to say that each tea has a characteristic odour.

Good-quality Assam tea that is lacking in strength would lose some cash value, merely because strength is an accepted feature of Assam tea. The strength of good Assam tea is almost quinine-like in its bitterness. Such high strength would be unacceptable to those who drink either Darjeeling or Ceylon tea, and would therefore tend to reduce the cash value of the tea. Thus the value of a tea depends on the market in which it is to be sold. Similarly, prices in auctions depend on a tea conforming to the attributes expected from its designation, as 'Assam tea', 'Ceylon tea', and so on.

These examples show that a certain odour, and a certain balance of quality and strength, define a kind of tea. Odour is decided in the first place by the botanical nature of the plant. It is to be noted that 'aroma', used in an earlier paper[1], was meant to be synonymous with 'odour' used here and by food technologists[6].

The kind of tea known as 'Assam' is made from *C. sinensis* var. *assamica*. It cannot be made from var. *sinensis*[7]. Hybridity with *sinensis* introduces unwanted odours; but hybridity with the southern form of plant seems to introduce desirable odours.

Darjeeling tea is made from populations with pronounced features of the type variety (var. *sinensis*). Some of the populations are hybrids between distinct species[8]. Odours of *sinensis* seem to be needed in Darjeeling tea[7]; but recent investigations strongly suggest that decisive odours have also been contributed by other taxa like *C. irrawadiensis* or *C. taliensis*, or even more distantly related species.

Plants of the Assam variety (var. *assamica*) grown in Darjeeling do not make Darjeeling tea. Representative Darjeeling plants, brought as seed

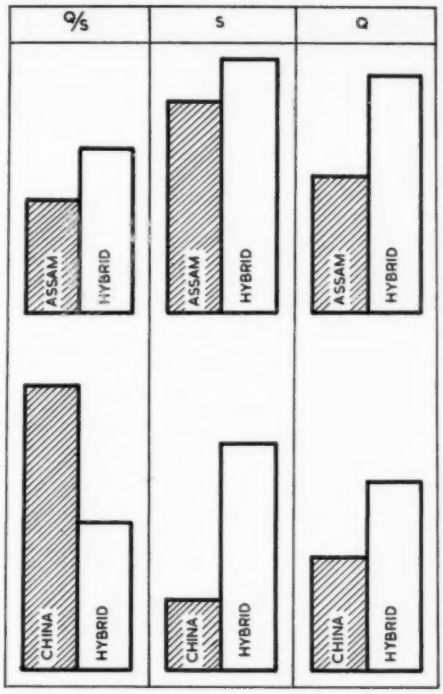

Fig. 1. Nature of the tea made from putative hybrids between *Camellia sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. Column heights indicate the tasters' perception of quality (Q) and strength (S), and the ratio of these measures (Q/S). 'Assam' and 'China' indicate plants not appreciably different from the respective types. 'Hybrid' means plants with some features of the other taxon, but assignable to the type with which they are coupled in the figure

to Assam, do not make Assam tea. Tea from similar plants, established in Assam as the descendants of seed imported directly from China and Formosa, has been accepted as Darjeeling tea by professional tasters.

Ceylon tea, like Darjeeling tea, is made from *sinensis* hybrids; but there are more features of the southern form in Ceylon than in Darjeeling[9]. It seems likely that genetical factors derived from the southern form play a large part in determining the characteristic odour of Ceylon tea.

It has not been found possible to make Assam tea from plants of the southern form growing in Assam; but open-pollinated seed of these plants, taken to high elevations in Ceylon, produced plants (of the southern form) that made good Ceylon tea. The possibility of making Ceylon tea in Assam has not been investigated; but the evidence clearly indicates that at least three kinds of tea, which take their names from regions, must be determined primarily by genotype and, possibly, by interactions of genotype with environment. Interactions in terms of 'phloem index' (which is associated with quality[1]) have been demonstrated in transplant experiments[10].

In addition to odour, the balance of quality and strength differs between taxonomic types. Fig. 1 shows that quality relative to strength is high in *sinensis*; conversely, high strength, relative to quality, characterizes *assamica*. Fig. 1 also shows that quality is improved by hybridity—a point that

was amplified recently[1]. The figure further shows that the strength of nominal *sinensis* plants is increased by slight hybridity with *assamica*; but, in this connexion, it is to be remembered that strength may not be particularly desirable in tea that can be made from *sinensis*. Comparable data for hybrids with the southern form are lacking, but the quality to strength ratio appears to be the same as that of *assamica*.

New kinds of tea could be a great asset to tea exporters; but it is evident that new odours would be needed. However, the odours in plants of the southern form are sufficiently variable to make them most desirable for breeding new kinds of tea. The form needs more study. It is the conceptual type[11] of material received from Tran-ninh by courtesy of M. du Pasquier who attached importance to the tea plants of that region[12]. Primary selection might lead to a stock as valuable to tea as the Barberton *U*4 strain was to cotton[13]. This would be due to odour. But the need, or otherwise, for strength, depending on prospective markets, would have to be kept in mind. Quality is always needed and is a concomitant of hybridity between distinct types[1]. In short, odour classification[6] and the separate definition of quality and strength seem essential to a progressive tea-breeding programme.

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NORWICH MEETING OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

IT can never be sufficiently stressed that the elaborate annual meetings of the British Association for the Advancement of Science, for which the Association is best known, are intended primarily to disseminate scientific knowledge among the lay public. The Association's one-hundred and twenty-third annual meeting: to be held in Norwich during August 30–September 6, may serve to underline this fact, for although Norwich is a historic provincial capital, it is the centre of a predominantly rural community in which scientific processes are not of the widest application. Furthermore, although in the near future it will be, Norwich is not at present a university town, so that it will not call on university resources to assist the meeting, as do the majority of the Association's hosts. Instead, it is calling on the staffs of the City College and schools in Norwich to help in organizing the sectional programmes, on schools and civic buildings to house the meetings and on its civic officers for the general organization.

The meeting will follow the customary pattern of an inaugural meeting: two evening discourses, a full programme of sectional lectures and excursions to many parts of East Anglia, some 45 excursions to places of general interest (in which the area is unusually rich) and a number of social functions, including four garden parties in settings which should prove ideal. There will also be a young people's programme which will include a 'Science in Schools' exhibition and an 'Open Forum' for both adults and young people at which scientists will answer questions impromptu. The presidential address to be given at the inaugural meeting by Sir Wilfrid Le Gros Clark will be entitled "The Humanity of Man".

There are, however, two features in the programme for the coming meeting which are novel. One will be a public demonstration of the new Swiss colour television technique known as Eidophor. Eidophor is a form of large-screen television projection invented by the late Dr. Fritz Fischer, of Zurich. Since his death in 1947 it has been continually developed by Prof. Baumann, and the costly and delicate apparatus used will be brought by road from London and installed in St. Andrew's Hall. It will relay to a smaller hall the proceedings at the inaugural meeting and on subsequent evenings will give demonstrations. This is likely to prove a fascinating feature of the meeting, and Norwich is indeed fortunate that it has been possible to arrange these demonstrations. The other novel feature is also in the sphere of the visual arts: this is a daily programme of scientific films from all nations which will be shown in the Noverre Cinema. There will be a different programme each day, and it is hoped that so comprehensive a collection of outstanding scientific films will prove a great attraction.

As is common, the majority of the branches of scientific study which are catered for at the Association's annual meetings are well reflected in Norwich and Norfolk. Norwich, by historic tradition, beginning with the growth of the the weaving industry, enjoys a great reputation as a home of handicraftsmanship. The decline of the medieval textile industries was offset by the craftsman's inherent versatility, and the change-over to the manufacture of shoes, in particular, during the past century, enabled the city to retain its reputation in this direction. In more recent times the development of light engineering has sustained native potential, and it is with this background that the subject of one of the discourses, "The Impact of Automation", to be given by Dr. B. V. Bowden, principal of the Manchester College of Science and Technology, assumes a particular interest. In the same way as physicists and engineers, the biologists, geologists and archæologists are provided with a fruitful research field, since East Anglia is a distinctive part of the insular land-mass of Great Britain. Once part of a terrain which was joined to the European continent and then largely inundated, its flora and fauna are objects for rewarding comparative study. The coasts and the Broads will be visited on many occasions both by people with specialized interests and those who wish to enjoy their scenic beauty. The subject of the other discourse, which

will be given by Field-Marshal Viscount Alanbrooke, will be "Birds of Spain", and this discourse, in an area notable for its wild life, is expected to draw a large audience. Archæologically, Norfolk's records of the historic ages prior to the Roman occupation are of absorbing interest, and of the Roman period itself they are probably unsurpassed in Britain.

Forestry and agriculture are perhaps the two sciences to which most justice can be done, since the Forestry Commission owns many acres in west Norfolk where afforestation is intensively pursued; and in the sphere of agriculture it would be hard to refute Norfolk's claim as the cradle of modern farming methods. From the time of Coke of Norfolk onwards, highly developed farming has been the major industry, and local agricultural experimental stations have made significant contributions to the progress of farming throughout the country.

With these factors in mind, and with the backcloth of a gracious cathedral city, the organizers of the Association's 1961 meeting hope that it will be even more successful than its predecessors in Norwich in 1868 and 1935. Every effort will be made to welcome visitors from outside the area and to ensure that they derive all possible pleasure and benefit from the meeting.

D. D. ALDRIDGE

OBITUARIES

Mr. J. Walter Ryde, F.R.S.

JOHN WALTER RYDE died at Marlborough, Wiltshire, on May 15, after a short illness. He was sixty-three years old. He was born in Brighton on April 15, 1898, the son of Mr. W. W. Ryde, and was educated at St. Paul's School and in France. Plans for him to continue his studies in Germany and then go to the University of Cambridge were interrupted by the First World War. He returned from Berlin to England just before the outbreak of war and entered the City and Guilds Technical College, Finsbury, where he studied under Prof. Sylvanus Thompson and Prof. Charles R. Darling.

Ryde joined the Royal Engineers voluntarily in 1916, at the age of eighteen, on the completion of his course at City and Guilds, and was trained in anti-aircraft work in Monmouthshire and on the Norfolk coast before being drafted to France during the last few months of the War. He continued to serve in France until his demobilization in 1919.

Ryde joined the Research Laboratories of the General Electric Company, Ltd., as a physicist in 1919, going directly from his war service to the newly established research unit directed by Clifford C. Paterson in the Company's lamp factory at Hammersmith. He was a leading member of the scientific staff of the Laboratories from the start.

In the early days at Hammersmith, and again after the Laboratories had moved to their new premises at Wembley in 1923, the variety of Ryde's research work was indicative of the breadth of interests which was so characteristic of him. They included pure spectroscopy, thermionics and the gaseous electrical discharge. Under his direction the first practical high-pressure mercury vapour lamp was developed—the foundation of a new industry to which he made many subsequent contributions.

His theoretical work on the scattering of light, first applied in the 'twenties to optical diffusing media in glasses, was extended by him during the Second World War to classic studies of the attenuation and the radar echoes produced by meteorological phenomena at centimetre wave-lengths. (A footnote in this paper includes the following: "The author (J. W. R.) and Mrs. Ryde were responsible for the theory and calculation of the effects caused by liquid and solid particles,——".) He was an early worker on crystal valves and quickly recognized the potentialities of germanium as a semiconducting material. Other researches included velocity modulation tubes, luminescent materials and optical projection systems. He contributed much to various research projects undertaken for the United Kingdom Atomic Energy Authority, and he took part in work on the *Fido* fog-dispersal system. It was typical of Ryde to design, in his leisure hours, a pocket illumination chart for use by the Services. It showed levels of illumination, by day and by night, at various latitudes, and was used extensively in military operations.

Ryde was elected a Fellow of the Royal Society in 1948. He was appointed chief physicist at the G.E.C. Research Laboratories—recently named the Hirst Research Centre—in 1950 and chief scientist in 1953. During these recent years he had overall responsibility for the programmes of pure scientific research of the Centre, and his particular interests lay in the fields of new materials, crystal growing and solid-state physics.

He was a member of the Royal Institution of Great Britain since 1935 and chairman of the Davy Faraday Laboratory of the Institution since 1951. He served on the Board of Managers of the Institution and always took a great interest in its affairs. He was a Fellow of the Institute of Physics and the Physical Society, a Fellow of the Royal Astronomical Society, and a member of the Geological Association, the Wiltshire Archæological Society and the Athenæum.

Ryde married, in 1930, Dorothy Ritchie, daughter of the then chief illuminating engineer of the General Electric Co. There is one son.

The Hirst Research Centre has lost a senior man who earned the admiration and respect not only of his colleagues but also of eminent men both in Great Britain and overseas. He lived his life for science, and his leisure activities were very largely extensions of his life's work. Archæology was a persistent interest—at an early age he learned to read hieroglyphic writing and ancient scripts. He was a profound student of astronomy, but, in recent years, microscopy became a dominant hobby. He was particularly interested in diatoms, and his beautifully mounted specimens are evidence of the elegant experimental skill which he retained to the end.

His memory was prodigious and visual. He never forgot the details of work which he had done and could sketch from memory some experimental arrangement or the detailed shape of a specimen long afterwards. Such a memory, supplemented with the many carefully prepared and indexed note-books which he compiled throughout his working life, made Ryde a powerful authority indeed in the world of science.

XUM

In all his work he displayed great ability and unfailing perseverance, together with brilliant insight and inspiration. His colleagues will always remember his patient readiness to discuss and assess the problems of the day and his helpful elucidation of critical experiments, often elegant in their simplicity, which he designed to solve them. O. W. Humphreys

Dr. P. A. Gorer, F.R.S.

The unexpected death of Dr. Peter Alfred Gorer on May 11 will be deeply regretted by all who knew him. Born in 1907, he was educated at Charterhouse before taking up medicine at Guy's Hospital. During this period he developed an interest in hibernation, and his earliest papers were on this subject. After qualification, he left Guy's for the Department of Genetics at University College, London, under J. B. S. Haldane. It was here, and later at the Lister Institute, that he first began a lifelong study of the genetic and immunological implications of tumour transplantation. At this time only the genetic basis of tumour transplantation had been established. Gorer proposed that genetically determined antigens present in neoplastic tissues were the immediate arbiters of the fate of such grafts. If the transplant introduced antigens not possessed by the recipient animal, rejection of the graft would follow. Between 1937 and the outbreak of the Second World War, he produced solid evidence in support of this idea. During hostilities he acted as assistant morbid anatomist, and hæmatologist, at Guy's Hospital, and remained afterwards as reader in the Department of Experimental Pathology. Only this year he had been chosen to fill the chair of a newly created Department of Immunology at Guy's Hospital.

On returning to the subject of his researches after the War, he spent several years developing the serological methods necessary before any further progress was possible. The defeat of these technical difficulties enabled him to perform a detailed analysis of the major system of tissue antigens in mice, known as the *H*-2 system. His latest paper on this subject indicated the progress which has been made in mapping the genes which dictate the presence of the *H*-2 antigens.

But Gorer was probably best known for his work on the part played by humoral antibody during the rejection of foreign tissue grafts. He established that such antibodies were present in the serum of mice within three days of receiving a graft of alien tumour; furthermore, normal tissues such as skin were similarly able to excite the formation of humoral antibodies. The function of these antibodies is still the subject of research, but it is clear from his evidence that humoral antibodies have several effects; which one predominates varies according to the target graft, and to a lesser extent the recipient.

At an early stage Gorer was aware of the probability of antigenic differences between normal and malignant tissues. The occurrence of antigenic loss had been implied by much genetic evidence, and he confirmed this by serological means. Few investigations distinguished clearly between normal tissue antigens possessed by tumour cells, and antigens peculiar to the neoplastic state. In 1956, in collaboration with one of his pupils, D. B. Amos, he discovered the first of a series of antigens specific to mouse lymphatic leukæmia cells. Later work showed that such antigens were detectable in spontaneous as well as induced leukæmias. It is sad indeed that he was unable to start on the important investigation of the significance of these antigens.

One cannot account for the pre-eminent position of Peter Gorer merely as a result of his brilliant researches and thoughtful reviews. He had great charm and wit, a breadth of interest rarely found, and perhaps the most striking attribute of all was an ability to discuss his own subject dispassionately. It gave great pleasure to his many friends and pupils when he was elected Fellow of the Royal Society in 1960. We shall greatly miss him.

He leaves a widow, a son and a daughter.

J. R. Batchelor

NEWS and VIEWS

U.S. National Academy of Sciences: Foreign Associates

The following have been elected foreign associates of the U.S. National Academy of Sciences: Prof. K. E. Bullen, professor of applied mathematics in the University of Sydney; Prof. B. Ephrussi, professor of genetics in the Sorbonne, and director of the Genetics Laboratory of the National Centre for Scientific Research at Gif-sur-Yvette; Dr. W. K. Heisenberg, director of the Max Planck Institute for Physics and Astrophysics, Munich; Prof. V. Prelog, professor of organic chemistry and director of the Laboratory, Federal Institute of Technology, Zurich.

Prof. K. E. Bullen, F.R.S.

Prof. Bullen is a native of New Zealand, born in Auckland in 1906. His interest in seismology was aroused by New Zealand's Hawke's Bay earthquake of 1931 and encouraged by Sir Harold Jeffreys, whom he met the same year, during Bullen's studies in Cambridge. Together they produced the Jeffreys–Bullen tables of seismic travel times, which have been used since 1940 in preparing the *International Seismological Summary*. Bullen's pressure-incompressibility hypothesis, issued in 1946, led to the inference that the Earth's inner core is solid (in the sense that it can transmit seismic waves), although surrounded by a fluid outer core; it also led him to construct his Earth model *B*, a widely used mathematical model of the Earth's seismic functions. In addition, Prof. Bullen is the author of several papers on the internal structures of Venus and Mars and on the origin of the Moon.

Prof. B. Ephrussi

Prof. Ephrussi was born in Moscow in 1901. On completing his secondary education there, he went to Paris in 1919 and continued his studies at the Sorbonne, where he received a Rockefeller Foundation fellowship and obtained the D.Sc. degree in 1932. During 1930–34 he undertook investigations in the field of chemical embryology and tissue culture,

becoming in 1931 an assistant to Prof. Faure-Fremiet at the Institut de Biologie Physico-Chimique. Among his numerous writings on these subjects, his book on tissue culture, published in 1932, is now an acknowledged classic. An interest in genetics, occasioned by his studies of tissue culture, led him to apply for a second Rockefeller Foundation fellowship to work with Prof. T. H. Morgan at California Institute of Technology during 1934–35. In the course of his experiments there in genetics and tissue culture, he observed that a certain combination of genes which causes death in embryonic mice is not necessarily lethal at the cellular level, since individual tissue cultures from these embryos will survive far beyond the embryo's ten-day life-span. In 1935 Ephrussi returned to France, accompanied by Prof. G. W. Beadle. Together they undertook a series of eye-disk transplantation experiments in *Drosophila* which resulted in the first experimental proof of the genetic control of biochemical reactions. After the Second World War he began his post-war studies on yeast, and soon discovered that mutations caused by acridine dyes transform aerobic into anaerobic strains. His subsequent classic investigations probed the biochemical interplay between cytoplasmic and nuclear elements, and resulted in a second book dealing with the significance of nuclear and cytoplasmic genetic variation in embryological development. He is now entering on a study of the genetics of mammalian somatic cells in tissue culture.

Prof. W. K. Heisenberg, For.Mem.R.S.

Prof. Heisenberg won the Nobel Prize for Physics in 1932, for the creation of quantum mechanics. His was a fundamental approach to the problems of atomic mechanics, based on observable physical properties such as the frequencies and intensities of spectral lines emitted by atoms and molecules; this system, known as matrix mechanics, dispensed with unwarranted assumptions inherent in earlier atomic theory, arising from pictorial representations of planetary orbits within the atom. In 1927 he solved the puzzle posed by the existence of two separate spectra of helium gas, showing that one of them arises when the two electrons of the helium atom are paired with opposite spin and the other when the spins are in the same direction. He then predicted, by analogy, that the hydrogen molecule should exist in two forms: one in which the paired hydrogen nuclei spin in the same direction, and another in which they spin in opposite directions. Two years later the two forms of hydrogen were experimentally shown to exist. It was also in 1927 that Heisenberg formulated his famous principle of indeterminacy (or uncertainty principle), which maintains that regardless of the nature of the experiment there is an inherent theoretical limit to the precision with which both position and momentum of a particle can be established simultaneously. He showed that this ultimate limit of physical measurement is defined by a fundamental universal constant that had been introduced into physics by Max Planck at the turn of the century.

Prof. V. Prelog

Prof. Prelog was born in Sarajevo, Yugoslavia, in 1906. He studied chemistry and chemical engineering at the Prague Institute of Technology, where in 1929 he received the degree of doctor of technical sciences. He headed the research laboratory of the G. J. Driza Co. in Prague until 1935, when he joined the Department of Technology of the University of Zagreb as an associate professor. In 1942 he joined the Department of Organic Chemistry of the Swiss Federal Institute of Technology (Eidgenossische Technische Hochschule) in Zurich, where he became associate professor in 1947, professor in 1950, and director of the Department in 1957. He is the author of some 270 scientific papers on such topics as alkaloids and steroids, heterocyclic and many-membered-ring compounds, antibiotics, and stereochemistry.

Applied Mathematics at Belfast: Dr. A. Dalgarno

Dr. A. Dalgarno, though only thirty-three years old, has been appointed to a chair of quantum mechanics in the Department of Applied Mathematics at the Queen's University of Belfast. He began his brilliant research career at University College, London, where in 1951 he obtained a Ph.D. degree. In the same year he moved to Belfast as an assistant lecturer, and was promoted successively to lecturer in 1952 and reader in 1956. Last year he was made director of the newly established Digital Computing Laboratory—an appointment he still holds. Since 1951 he has paid a number of prolonged visits to the United States as guest research worker at the Massachusetts Institute of Technology, and as consultant to Air Force Cambridge Research Center and to the Geophysics Corporation of America. Dr. Dalgarno's research interests include the methods of quantum mechanics (especially perturbation theory), atomic and molecular structure, collision processes and upper atmospheric physics. His skill at mathematical analysis is exemplified by his classical paper with Dr. J. T. Lewis (*Proc. Roy. Soc.*, A, **233**, 70; 1956) on the calculation of long-range forces between atoms. This paper gives the exact solution to an important problem which had been treated by approximate methods for more than a quarter of a century. As well as being very creative, Dr. Dalgarno is a stimulating supervisor of research students. His appointment will consolidate the strength of the postgraduate school at Belfast.

Machine Tool Engineering at Manchester: Prof. F. Koenigsberger

Dr. Franz Koenigsberger has been appointed to the newly created chair in machine tool engineering in the Faculty of Technology in the University of Manchester. Dr. Koenigsberger received his engineering training in the Technische Hochschule, Berlin-Charlottenburg, where he qualified for the degree of Dipl.Ing. in 1931 with honours. He obtained the degree of M.Sc.Tech.(Manchester) in 1952 and the degree of D.Sc.(Manchester) in 1954. After a period of research experience under Prof. Schlesinger in Berlin, he obtained industrial experience in Berlin and later in Belgium. During 1935–38 he was chief engineer and chief designer in the Machine Tool Department of Ansaldo, Genoa, and in 1939 became chief mechanical engineer for Messrs. Cooke and Ferguson, Ltd., a post he held until he was appointed lecturer in mechanical engineering in the Manchester College of Science and Technology in November 1947. He was appointed senior lecturer in production engineering in 1955, and reader in machine tools and production processes in May 1957. Dr. Koenigsberger is well known for his work in the field of production engineering, and is a member of the International Institution for Production Engineering Research.

XUM

Medical Statistics at Belfast: Prof. E. A. Cheeseman

Dr. E. A. Cheeseman was recently appointed to a personal chair of medical statistics in the Department of Social and Preventive Medicine at the Queen's University of Belfast.

Dr. Cheeseman was educated at the William Ellis School and the University of London. He obtained his B.Sc. (Economics) in 1938, and Ph.D. (Medicine) in 1947. He was a member of the Territorial Army at the outbreak of war and until early 1945 served with the Royal Artillery. After six months as a statistical staff officer with H.Q. 21 Army Group he was demobilized with the honorary rank of Captain. After the War he joined the Department of Epidemiology and Vital Statistics at the London School of Hygiene as a member of the Medical Research Council's Statistical Research Unit under Prof. A. Bradford Hill. In 1948 he became lecturer in medical statistics in the Department of Social and Preventive Medicine at the Queen's University of Belfast, and adviser in medical statistics to the Northern Ireland Hospitals Authority. He was appointed reader in 1952. He is a member of the Statistical Committee of the Medical Research Council and of the Statistics Sub-Committee of the Registrar-General of England and Wales, and has been the author or part-author of numerous publications in the field of human genetics and the statistical aspects of medical research. His chief professional interests are advising medical colleagues on the design and analysis of experiments and trying to convince medical undergraduates and postgraduates that statistics are not as difficult as they appear to be.

Control of Colleges of Advanced Technology in Britain

On June 22, Sir David Eccles, Minister of Education, made a statement in the House of Commons on the development of colleges of advanced technology. Control of these colleges, which have been maintained by local education authorities, should be transferred to independent governing bodies. These would receive direct grants from the department of education. His proposal stems from the observation that the success of these colleges in developing as national institutions of higher education has engendered new problems of planning and staffing. Though local government has given good service in the past, to-day the colleges are too big for local management. Bearing in mind the quest of the Robbins Committee and the consideration it might be giving to the future of colleges of advanced technology, the Minister is writing to associations of local authorities and teachers to propose this new status. To preserve links with the local authorities, who have been so helpful, it is suggested that the authorities continue to be represented on the new governing bodies.

The Colonial Microbiological Research Institute, Trinidad

The Colonial Microbiological Research Institute, Port of Spain, Trinidad, was opened on July 5, 1948, by Lord Hankey, chairman of the Colonial Products Research Council. The function of the Institute was to devote its energies to fundamental research in microbiology, to the application of the results of its investigations and to assisting agricultural and other industries in improving their products. The Institute maintained a collection of micro-organisms, the Hankey Culture Collection, in which microbes of industrial or agricultural importance received special attention. It has now been decided to close the Institute as from April 30. It had been financed from Colonial Development and Welfare Research funds up to March 31, 1960, and by the Government of Trinidad and Tobago during the past year. The buildings had become the property of the Government of Trinidad and Tobago. They have since been given to the University College of the West Indies and leased by the College to the Trinidad Regional Virus Laboratory in furtherance of closer association between these two institutions. The Hankey Culture Collection is now housed in the Faculty of Agriculture, University College of the West Indies, St. Augustine, Trinidad, and is under the charge of a curator.

Science and Television

The first attempt to provide a connected and long-term series of science programmes on television for adults has been announced by the B.B.C. Broadcast on Saturday mornings, the series has been grouped under the headings of "The Structure of Matter", "Physics in the Service of Man", "Human Biology" and "Science for Medicine", and will continue until the end of the year. Each programme has been broadcast previously on one or other of the B.B.C. programmes, but they have been re-arranged to provide a new basis for study and to illustrate definite themes. The speakers include many well-known men of science from Great Britain and the Commonwealth, and should enrich a collection of programmes which should bring pleasure to many viewers. It will be interesting, later, to see which members of the community have followed the broadcasts. Full details of the whole series have been set out by the B.B.C. in a recent pamphlet entitled *Saturday Morning Television introduces Science on Saturday* (Pp. 16. London: British Broadcasting Corporation, 1961), which can be obtained from 35 Marylebone High Street, London, W.1.

Pfizer Virus Research Laboratories

The latest aid to vaccine research by the Pfizer Group at Sandwich is the provision of a new specially designed building for research into virus diseases. It has extensive laboratory and animal accommodation, and is designed to preclude the possibility of infectious organisms escaping to the outside as well as preventing any contamination entering the building. The unit consists of three sections: a group of laboratories and two groups of animal blocks of four wards each, all completely separate and designed so that movement of personnel and air between each is controlled. Each animal ward and laboratory has its own high-intensity ultra-violet lock entrance. Sterile air is supplied to individual rooms and temperatures can be adjusted individually. One of the most important research projects to take place in this building will be the investigation of trachoma, a virus disease which causes blindness in Africa and Asia. The Pfizer Group are co-operating with the British Medical Research Council's Trachoma Research Group in this project.

Strontium-90 in Milk, 1960

A number of investigations have already indicated that the levels of strontium-90 in milk produced in the United Kingdom have been determined in large measure by the rate of fall-out occurring at or near

the time of milk production. Consequently, a fall in the rate of deposition should result in a downward trend of the activity-levels in milk. This is borne out in a report by the Agricultural Research Council on the strontium-90 levels in milk samples, representative of a substantial fraction of United Kingdom milk-production during the period January–June 1960 (Report No. 4, *Strontium-90 in Milk and Agricultural Materials in the United Kingdom*, 1959–1960. Pp. xiii + 81, 34 tables, 14 figures. London: H.M.S.O., 1961. 5*s*.). Thus it appears, on one hand, that the deposition in the first six months of 1960 was less than 25 per cent of that in the same period of 1959, and, on the other, that the strontium-90 levels in milk at June 1960 were about one-half of those observed twelve months previously. Complete information on strontium-90 levels for other items of the diet in 1960 is not yet to hand, but the milk results give a preliminary indication that the mean level in the total mixed diet of the population was appreciably lower for 1960 than for the previous year. In addition to a full discussion of the milk results, the report assesses the results of a survey of strontium-90 in permanent pastures and animal bone carried out during 1959. It also includes useful summaries of the results of experimental investigations designed to elucidate the factors controlling the passage of fission products through food chains. Throughout, the discussion is lucid, and the experimental results are well presented.

Technical Education Abstracts

THE place of technical education in the national economy is now so fundamental that any journal devoted exclusively to its promotion deserves the warmest of greetings. In the case of the newly launched *Technical Education Abstracts*, however, some qualification is necessary. (The National Foundation for Educational Research in England and Wales. *Technical Education Abstracts from British Sources*, September–December, 1960 (Vol. 1, No. 1). Pp. 57 + xxviii. (London: National Foundation for Educational Research in England and Wales, 1961.) Subscriptions: 42*s*. per volume of four quarterly issues.) Many of the abstracts necessarily relate to articles which are largely persuasive, and, though compelling interest at the time of publication, lack much of the force of the original articles both because of the condensed form and because they refer to topics which may have lost their urgency. This criticism may be offset if the publishers can, as they suggest, reduce the time between abstraction and the date of publication. A further improvement could be made in the selection of abstractors. Few of these are known in the field of technical education and this may explain why so many of the abstracts are taken from relatively few journals. The range could be widened if more help were obtained from industrial correspondents, many of whom would give their services voluntarily.

History of Medicine and Pharmacy at London

THE Faculty of History of Medicine and Pharmacy was founded under the auspices of the Worshipful Society of Apothecaries of London (which received its Charter from King James I in 1617) on April 3, 1959. The aims of the Faculty are to foster and extend more general interest in medical history by enlisting the support of all medical corporations, schools, societies, clubs and individuals. The Faculty also welcomes the interest and support of general historians in forging closer links between medical and pharmaceutical history and general, social and economic history. The Society's ancient Hall in the City of London serves as a general meeting place for lectures, discussions and other meetings held in pursuance of these aims. In the second annual report (*The Worshipful Society of Apothecaries of London: Faculty of the History of Medicine and Pharmacy. Second Annual Report and List of Members*, 1960–61. Pp. 20. London: The Worshipful Society of Apothecaries of London, 1961) the Faculty describes some of the considerable progress during the past year. Membership now stands at 304, and, on the initiative of the Faculty, St. Thomas's Hospital Medical School has appointed "a Society of Apothecaries Lecturer in the History of Medicine", and the governing body of King's College Medical School has already approved the establishment of a similar appointment. The Faculty's endowed lectures have now been named: the annual students' lecture will henceforth be known as "The Osler Lecture", and the autumn lecture will be named "The Sydenham Lecture", after the great English clinician of the seventeenth century, Thomas Sydenham. Dr. Kenneth Dewhurst has accepted an invitation to give the first "Sydenham Lecture" on November 22, 1961, at Apothecaries' Hall; his subject will be Thomas Sydenham. The second British Congress on the History of Medicine is to be held in London during September 28–29.

The Library Association

THE annual report of the Council of the Library Association for the year ended December 31, 1960, presented at the annual meeting in Hastings, 1961, describes the steps taken to press for early action by Parliament on the proposals of the Roberts Committee for the reform of the public library service (Pp. 28. London: Library Association, 1961). A memorandum on the status and training of reference librarians has been adopted by the Council and distributed to all chief librarians, and, at the request of the Select Committee on Estimates, a memorandum regarding the House of Commons Library was presented to the Select Committee, and a deputation later gave oral evidence. An investigation was initiated into ways and means of helping the developing Commonwealth countries, particularly in Africa and Asia, to establish more adequate library services. As a first step a survey is being made of existing services in these countries. A comprehensive and detailed guide to British periodicals is being compiled, and suggestions for improving the classification and expansions to the schedules have been sent to the editor of the Dewey Decimal Classification. The Association is taking steps to establish a National Advisory Council for Micro-reproduction following a meeting at which all types of libraries, learned societies and institutions were represented; a steering committee has been appointed.

The Brown Library, Liverpool

AN illustrated booklet issued by the City of Liverpool Libraries, Museums and Arts Committee as a guide to the rebuilt Brown Library and the new links with the Picton and Hornby Libraries gives a concise history of the Brown Library, and then describes briefly the services which the rebuilt library offers in commerce, science and technology (Pp. 32. Liverpool: Brown, Picton and Hornby Libraries, 1961). Besides the special research services to science and industry offered by Ladsirlac, these include a

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commercial and social sciences library, technical library, science library, technical documents unit, patents library and microfilm unit. In the arts the library includes a Commonwealth library, an American library, international library, biography library, reference issue foyer, and a record office and local history library. The booklet displays well the regional character of the libraries and their services, and it should be noted that they are depository libraries for the publications of the American, British and European Atomic Energy Commissions, and for the publications of the United Nations Organization and the United Nations Educational, Scientific and Cultural Organization.

Library of the U.S. Naval Observatory

An article by Marjorie S. Clopine, librarian of the United States Naval Observatory, reprinted from *Special Libraries* (52, No. 2, February 1961) and issued by the Observatory as Reprint No. 19, describes the resources and treasures of the Library, which are of particular interest for astronomers. By 1960 the Library contained more than 55,000 catalogued volumes and also many sets of periodicals, the mathematics collection being especially strong in analysis and noted for its value in reference work. There is a collection of 500 rare books printed before 1800. Besides long sets of nautical almanacs, the Library has a valuable collection of star catalogues and charts, observatory publications, bibliographical reference works in astronomy, the complete works of many astronomers and mathematicians, and mathematical tables.

Chemical and Allied Industries at the Science Museum Library

Under the title *Books on the Chemical and Allied Industries* the Science Museum Library has issued, for the Ministry of Education, a short-title list of books published in 1930 and onwards on the chemical industry, with books on mining and metallurgy, and the paper, leather and textile industries and received in the Science Library up to the end of 1959 (Pp. ix + 118. London: H.M.S.O., 1961. 12*s*. 6*d*. net). The list has been compiled by L. R. Day from the subject catalogue in the Library and is the second in a series of catalogues, each carrying a large division of the Science Library's collection. Entries are arranged in broad subject groups selected from the Universal Decimal Classification, and both an author index and a subject index are included. While intended primarily to enable users to take advantage of books available in the Library it is hoped that the list will assist other libraries in both the selection and classification of works in these fields. A Supplement, issued as Science Library Bibliographical Series No. 781, includes books received in the Science Library in 1960 (Pp. 10. London: Science Museum, 1961).

New American Electricity Plant

The largest power plant in the western hemisphere has recently begun to operate on the United States–Canadian border. It harnesses the power of the River Niagara, which drops 325 ft. over cataracts and falls in its 34-mile course between Lake Erie and Lake Ontario. The new hydro-electric development, one of the world's largest, is the Robert Moses Niagara Power Project. The power output of its two generating plants is 2,190,000 kilowatts, exceeding the giant United States' Grand Coulee Dam. The new Niagara River project began power production three years to a day after contracts for its construction were signed. Construction has been rushed and limited operation started because of a power shortage that has existed since 1956, when a power plant was destroyed by a rock-slide at Niagara Falls. Only two of the plant's 13 generators are now operating, but the remaining 11 will be completed and placed in use at the rate of one each five months. The Robert Moses Niagara Power Project, built by the New York State Power Authority and named after its first chairman, is a 720,000,000-dollar non-profit undertaking, financed through the sale of bonds to private investors. Cost of the plant and interest to investors will be paid through the sale of electricity. Several utility companies have contracted to buy the plant's electrical output. Because of limitations of water-diversion, the United States' share of river-flow during daylight in summer is not sufficient for continuous operation of the new plant. To ensure a constant water supply, the Robert Moses project includes a 1,880-acre storage reservoir which will be filled through diversion of Niagara River water during the winter. The reservoir, however, is more than an alternative source of water for operation of the power plant. It has been equipped with a reversible pump-generating system, and is used for production of 240,000 kilowatts of electricity.

Coal Tar Technology

Review of Coal Tar Technology, for the first half of 1960, presents the customary authoritative survey of international research, development, production, purification and patents relative to coal tar and its products covering that period. (January–June, 1960, 12, Part 1. Pp. ii + 63. Gomersea, Leeds: Coal Tar Research Association, 1960. 20*s*. per volume.) Section 1 reviews the then current work on coal tar itself, the primary fractions light oils, benzole, pitch and bitumen; tar acids; tar bases; monocyclic and polycyclic aromatic hydrocarbons; also utilization, which ranges from road tar, paints, pipecoatings, solid and liquid fuels, lubricants, plastics and resins, to wood preservatives and pesticides. Section 2 is devoted to general chemistry and chemical technique of coal tar and products; space is taken up chiefly with catalytic processes, for example, alkylation, isomerization, hydrogenation, oxidation, desulphurization, cracking. Physical chemistry of these substances concerns mainly rheology, vapour pressure and thermal properties. The paragraphs on analytical work include spectroscopic and chromatographic applications to coal tar and products, while a concluding category of chemical techniques describes recent work in connexion with distillation and crystallization problems.

Glossary of Petroleum Terms

The *Glossary of Petroleum Terms*, first published in 1951, with a second edition in 1955, has now been revised and enlarged, bringing it up to date. (Third edition, revised and enlarged. Edited by George Sell. Pp. vii + 39. London: Institute of Petroleum, 1961. 5*s*.) The petroleum industry is no exception to the trend of many others in developing over the years of its evolution a colloquial vocabulary understood only by those engaged in its day-to-day pursuits. In fact, especially in English-speaking international environments, the particular 'jargon' involved is, to the layman, probably more incomprehensible than in the case of many other modern, comparable commercial undertakings. Such words

as 'bean', 'bund', 'Christmas tree', 'feedstock', 'fishing', 'go-devil', 'overhead', 'packer', 'sweating', 'wildcat', to quote only a few, have quite different meanings in this industry from those ordinarily understood in general conversation. There is need for such specialized guidance as this, especially for the non-technical people in the industry, but this diminutive pamphlet (it is scarcely more than that, for of its 39 pages, 29 are devoted to the 'glossary' itself, 7 to an appendix on petroleum terms in U.K. legislation, 3 to abbreviations of titles of some official bodies, for example, API, DTD, IP, NCB, SAE, etc.) provides only modest fulfilment. Petroleum technology to-day is such an enormously ramified subject that nothing short of a comprehensive dictionary devoted to its tenets can cover the ground of its words and meanings adequately. The task has been attempted elsewhere some years ago, but even then on a rather limited scale. Whether the time is now ripe for conception and execution of a bold enterprise of this nature is a matter of judgement, policy and cost, but it is one to which the Institute of Petroleum, responsible for this *Glossary*, might well give careful consideration before the next edition is due for publication.

Improving Biological Communications in the United States

Establishment of a Biological Serials Record Centre is a principal recommendation of the Biological Communications Project, supported by the National Science Foundation, with the objective of collecting and disseminating detailed information on all biological serial and periodical publications, estimated at 25,000–50,000. The Centre will assess what proportion of this material is available to biologists in the United States, and will also study the general effectiveness of present periodicals as a system of communication. The National Science Foundation has also made a grant of 56,000 dollars for a one-year study of chemical notation systems, to be conducted by the National Academy of Sciences–National Research Council, and is supporting for a second year the Chemical Abstracts Service of the American Chemical Society's project to provide a centralized information service for chemists. A survey of computer systems for searching chemical literature has been completed by the National Bureau of Standards Research Information Centre and Advisory Service on Information Processing, and its results are available in a report by E. C. Medam and H. R. Koller, obtainable at 2.25 dollars from the Office of Technical Services, U.S. Department of Commerce, Washington 25, D.C.

Eoliths and Prehistorical Tools

As far back as 1911 there were furious quarrels among British prehistorians as to whether or not the Tertiary eoliths found by Reid Moir were really fashioned by man. To some extent the quarrel was theological: Was it conceivable that mankind could be as old as was suggested by the finds? Much research was undertaken, and at the time it was determined that Nature could chip, the blows coming from one or many directions, but not from two, or, better, three directions only. Further, if a piece of tabular flint was boldly chipped along one edge and then the arêtes between the flake scars carefully removed by subsequent flaking so as to produce a sharp, flaked edge, then Nature could not have been responsible for the work. Briefly, if it were possible to demonstrate design then the chipped object had been fashioned by man. Later the study of prehistory spread to other continents, but the above criteria were largely forgotten. Much chipped material claimed to be man's handiwork was unearthed and this material in turn became the yardstick for the study of still more flaked objects. Now the matter is being carefully reconsidered, and Dr. Desmond Clark has undertaken considerable research work, especially as regards materials other than flint. In a recent monograph (*Bull. Brit. Mus.* (*Nat. Hist.*), 5, No. 4. Pp. 71–90 + plates 15–23. London: British Museum (Natural History). 17*s.* 6*d.*), Dr. Clark concerns himself with specimens of very early Pleistocene age from certain beds in Israel. His conclusions are that probably every one of the specimens in question has been flaked by natural agencies, and these agencies he describes in some detail. Of course, should man have been in existence at the time in what is now Israel there was nothing to stop him picking up a naturally chipped object and using it as a tool. But that is not the question under consideration. The article has a wider interest than merely whether certain specimens from Israel were fashioned by Nature or by man; the detailed studies of how Nature can work will be very useful to prehistorians in other areas when they have to determine whether or not their early finds are really genuine tools or *lusi naturae*.

The Institute of Fuel

Dr. James Burns, deputy chairman, North Thames Gas Board, has been elected president of the Institute of Fuel, and will take office in October. Sir Harold Hartley has been made an honorary member of the Institute. Sir Harold was chairman of the Fuel Research Board of the Department of Scientific and Industrial Research during 1932–47; chairman of the British National Committee and of the International Executive Council of the World Power Conference during 1935–50; and president of the Conference during 1950–56.

Commonwealth Scientific and Industrial Research Organization Chemical Research Laboratories

Following the appointment of the director of the Commonwealth Scientific and Industrial Research Organization Chemical Research Laboratories, Dr. I. W. Wark, to the Executive, it has been decided that, in future, overall management of these Laboratories will be the responsibility of a committee of the chiefs and officers-in-charge of the various divisions and sections at Fishermen's Bend, Melbourne. The first chairman is Dr. A. L. G. Rees, chief of the Chemical Physics Division.

A New Computing Centre

On June 7 the IBM Co. opened a new data-processing centre at 58 Newman Street, London. This centre is equipped with type 7090 and type 1401 computers; the former machine is one of the most powerful at present in operation, and the latter forms a suitable second echelon support device. Users of the centre are expected to do their own programming, but this is relatively easy because the *Fortran* automatic programming system is available and this is backed by the *Share* user co-operation system. The centre staff will be available for basic training and also to give advice to users in particular cases.

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The Selby Fellowship

The Selby fellowship has been established to bring graduates of high promise and proved capacity for research to work in the laboratories of universities or research institutions in Australia. The fellowship may be awarded in any branch of physical or biological science, and is tenable at any university or research institution in Australia, normally for one full year of work. Candidates must have taken their first university degree, and have had preliminary research experience, at some university or universities in a country other than Australia. In general, they should hold a Ph.D. degree or have equivalent status and experience, but the fellowship will not normally be given to those who have already held a senior research award. Applicants should be less than thirty years of age on July 1 in the year in which his application is received. The fellowship will carry a stipend of £1,500 (Australian) per annum. The costs of the Fellow's travel to Australia, and back to his country of origin at the conclusion of the fellowship, will be provided, up to a limit of £A500, including dependants, if any. Applications should be sent to the Assistant Secretary, Gordon Street, Canberra City, A.C.T., before July 30.

Challenger Society Grants for Marine Biology or Oceanography

The Challenger Society has announced that it is prepared to make small grants for research in marine biology or oceanography at a recognized laboratory during the year June 1961–62. Applications, accompanied by details of the proposed research, should reach the Honorary Secretary, Challenger Society, National Institute of Oceanography, Wormley, Godalming, Surrey, not later than July 14.

The Training of Biochemists

The Biochemical Society is organizing a colloquium on "The Training of Biochemists", on July 13, during the course of the Society's two-day meeting in Oxford (July 13–14). Included in the programme will be the following topics and speakers: biochemistry as an honours degree subject (Prof. S. V. Perry); the graduate biochemist and industry (Dr. W. F. J. Cuthbertson); university and departmental entrance requirements (Dr. Margaret Kerly); the teaching of biochemistry at Oxford (Prof. D. D. Woods); teaching in a small non-professorial department (Dr. G. R. Tristram); the M.Sc. course in biochemistry (Dr. E. M. Crook); summarizing remarks (Prof. F. C. Happold). Further information can be obtained from Dr. K. S. Dodgson, Department of Biochemistry, Physiology Institute, University College of South Wales and Monmouthshire, Newport Road, Cardiff.

Announcements

Dr. W. E. Swinton, of the Department of Palæontology, British Museum (Natural History), South Kensington, has been appointed head of the Division of Life Sciences at the Royal Ontario Museum, Toronto; Dr. Swinton will take up his new appointment in September.

Mr. B. L. J. Hart has succeeded Mr. Frank Worsfold as principal of the Computer Application Training Centre, of International Computers and Tabulators, Ltd., at Bradenham Manor. Mr. Hart was formerly a member of the International Computers and Tabulators' Computer Group, and is well known for his work on management and production control.

The British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society are holding a joint conference on "Rheological Techniques" in Llandudno during September 20–22. Further information can be obtained from Dr. Peter Lord, Royal College of Advanced Technology, Peel Park, Salford 5.

An international conference on "Embryology", the fifth of its kind, is being sponsored by the editorial board of the *Journal of Embryology and Experimental Morphology*, and will be held in London at the Middlesex Hospital Medical School during September 18–21. Further information can be obtained from Dr. L. Brent, Department of Zoology, University College, Gower Street, London, W.C.1.

Jointly the Science Council, the Physical Society and the Crystallographic Society of Japan are organizing an international conference on "Magnetism and Crystallography" at Kyoto during September 25–30. Further information can be obtained from either Prof. T. Nagamiya or Prof. S. Miyake, Organizing Committee of the International Conference on Magnetism and Crystallography, Science Council of Japan, Ueno Park, Tokyo, Japan.

An informal conference organized jointly by the European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, on "Fission and Spallation Phenomena and their Application to Cosmic Rays", will be held at the Organization's Laboratories, Meyrin (Geneva), during September 26–29. Further information can be obtained from the Scientific Conference Secretariat (E. W. D. Steel), CERN, Geneva 23, Switzerland.

The Mass Spectrometry Panel of the Institute of Petroleum, in conjunction with the American Society for Testing Materials (Committee *E*-14), is organizing a conference on "Mass Spectrometry" at the University of Oxford during September 12–15. Further information can be obtained from the organizing secretary, Mr. W. J. Brown, Instrumentation Division, Associated Electrical Industries (Manchester), Ltd., Trafford Park, Manchester 17.

The University of Chile and various societies of Santiago and Valparaiso are organizing an international symposium on "Tissue Transplantation", to be held in Santiago and Valparaiso, Chile, during August 30–September 2. The programme will cover: the genetical basis; immunological problems; present status and prospective applications of tissue transplantation. Further information can be obtained from Dr. Alberto P. Christoffanini, secretary of the organizing committee of the symposium, Maximo Humbser 567, Santiago, Chile.

Errata. In the article on "Technical Assistance to Overseas Countries" in *Nature* of June 17, p. 1039, par. 4, lines 5 and 6, for "directorates of the Overseas Services and the Overseas Geological Surveys" read "Directorate of Overseas Surveys and the Overseas Geological Surveys".

The sixth International Nematology Symposium will be held in Ghent during July 24–28. It is regretted that on p. 963 of the June 10 issue of *Nature* the word "Hæmatology" was printed for "Nematology".

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NEW RESEARCH LABORATORIES FOR THE PAINTS DIVISION OF IMPERIAL CHEMICAL INDUSTRIES LTD.

IT is no exaggeration to say that, within the past twenty-five to thirty years, a revolution has taken place in the paint industry which has changed paint-making from an art into a science. As a result, the simple linseed oil/natural resin paints which had been in use for centuries have been displaced almost entirely by a wide range of synthetic coatings, each of which is specially formulated for the conditions under which it will be applied, and for the performance it will have to give, whether this is on a house, a high-speed aircraft, a motor-car, a household appliance or on one of the many other surfaces to which paints and lacquers are now applied.

This rapid development of modern paint coatings is the result of the interplay between the increasingly stringent requirements of the users and the research and development work carried out within the industry to anticipate these needs. The applied research necessary to produce the required products has in turn been nourished and stimulated by the fundamental research on polymers carried out within the industry and at universities and research institutions, and by the increasing range of synthetic raw materials that have become available from the chemical industry.

The Paints Division of Imperial Chemical Industries Ltd., which was one of the first paint companies in the United Kingdom to establish a research department some thirty years ago, has always placed considerable emphasis on research directed towards improving its existing products and to the production of new ones. To improve the accommodation for its Research Department, a five-storey block of laboratories has been erected recently on the Division's headquarters site at Slough, Bucks.

The new building, which is 48 ft. wide × 160 ft. long, provides about 30,000 sq. ft. of working space for laboratories, offices and the library of the Division.

It is constructed of *in situ* concrete faced with Leicester golden brown and Vandyke brown bricks, ornamented with blue mosaic column facings and artificial stone copings, and is planned on a 5-ft. longitudinal module. On all floors, the main corridor has been offset from the centre line, giving 22-ft. wide rooms on the north side and 15-ft. wide rooms on the south side. On the lower floors, where it is unlikely that the function or size of rooms will need to be altered, the walls are generally of solid construction; but on the higher floors, where changes are more likely to occur, 'Holoplast' partitioning has been used.

Fig. 1. North and west sides of I.C.I. Paints Division's new research laboratories at Slough, Bucks., showing the entrance and library reading room on the left

Floors generally are covered with large grey linoleum tiles finished with a polyurethane lacquer. The 5-ft. spacing of the columns on the outside walls has allowed for the provision of large sash windows, and the false ceilings, above which are carried the horizontal service distributions for the floor above, are rebated on the window side to increase the window height to the maximum.

Space heating is carried out by means of hot-water convectors built into the walls below the window sills. Window ventilation is relied on for offices, but in the laboratories, the fume-cupboard system provides the main ventilation. The fume-cupboard fans are run continuously throughout the day, warmed replacement air, cleaned by electrostatic precipitation, being supplied to all laboratories through a system of trunking concealed in the corridor ceiling.

To enable the extract ducts from fume cupboards on the lower floors to be carried up to the roof without interfering with the design of laboratories on the upper floors, the majority of the fume cupboards are recessed into a 4-ft. wide longitudinal strip which has been provided on all floors between the corridor and the laboratories on the wide side. Vertical ducts located in this strip carry the extract trunking from the different groups of fume cupboards up to the exhaust fans on the roof. Similar ducts carry the main risers for the piped and electrical services and the laboratory drainage. Access to all these ducts and to the backs of fume cupboards is provided from doors in the corridor, thereby minimizing interference with work in the laboratories when maintenance is necessary.

In general, 5-ft. wide double-sided island benches at right-angles to the windows have been adopted as the standard with a 5-ft. walk-way between, giving a 10-ft. centre-to-centre spacing between benches, corresponding to the 10-ft. tap-off systems on the ring mains and the 5-ft. columns on the window walls. With the present staff, this type of layout provides an average of about 15 ft. of bench per person. A special feature of the benches is the grouping of the service outlets into towers. Electrical sockets are mounted vertically on the fronts of these towers, with the water-control valves immediately above them, the corresponding water outlets being located at the side of the towers over a central lead-lined trough drain. The outlets for gas services (town gas, compressed air or nitrogen) are at the base of each tower, with extended control spindles to handles mounted on the fascia of the bench. The tops of the towers are recessed to carry the ends of over-bench shelves, beneath and on each side of which are mounted lengths of 'Unistrut' channel. On these slide specially designed clamps for the erection of bench scaffolding, thus eliminating the need to use retort stands for the support of apparatus. Similar 'Unistrut' channels have been provided on the walls in many laboratories for the erection of tall apparatus, and in these locations wall service outlets have also been provided.

Bench tops are either of teak, stainless steel or 'Formica' according to the particular type of work being carried out on them. The underbench furniture is of painted softwood and consists of 3-ft. 4-in. long interchangeable cupboard and drawer units.

The laboratories on the top floor of the building are devoted to organic chemical work directed towards the discovery of new polymers which may be of value as surface coatings, while those on the second and third floors are concerned with the further development of these polymers into paints and with their evaluation in comparison with standard products. The second floor also provides accommodation for the research management, administration, a central typing service and a conference room. The first floor is almost entirely occupied by the Physical Chemistry and Analytical Sections, both of which make extensive use of physical techniques such as infra-red and ultra-violet spectroscopy, polarography and chromatography, etc. The Physics Section, which is housed on the ground-floor, is concerned with problems involved in the dispersion and physical behaviour of pigments and with the physical characteristics of paint films and their effect on such properties as gloss, hardness, adhesion and durability. The remainder of the ground-floor is occupied by the Division Library and Information Service, which, in addition to housing a library of some 20,000 volumes dealing with a wide range of scientific interests, provides information in the form of bulletins, literature surveys, translations, etc.

The basement, as well as housing the main engineering services for the building, also provides accommodation for two air-conditioned rooms used for physical tests on paint films.

J. W. Dorling

NORTH BORNEO EXPEDITION 1961

MOUNT KINABALU (13,455 ft.) is the highest peak between the Himalayas and the Snow Mountains of New Guinea, and, though not a snow-mountain itself, it has an alpine flora relating these two parts of the world. It was first climbed in 1851 by Sir Hugh Low and has since attracted many botanists, zoologists and geologists. It is now known to have one of the richest floras of any mountain, but a great part of it remains to be explored.

To further the biological exploration of North Borneo, which will also assist the Government of North Borneo in its plans for the establishment of a national park on this famous mountain, the Royal Society set up in 1960 a committee under the chairmanship of Sir Joseph Hutchinson. This year, in co-operation with the Government of North Borneo, the Society has sent out a reconnaissance expedition under the leadership of Mr. E. J. H. Corner to investigate the practically unexplored eastern side of the mountain, where the forest extends down to the lowlands. In particular, the vegetation and soils are being studied as indicators of land which should be conserved within the boundaries of the national park. It is well known that in these precipitous regions of high rainfall, the clearing of luxuriant primary forest may lead to irreparable impoverishment of the soil with erosion and consequent silting and flooding of the rivers.

As soil-scientist, Mr. G. P. Askew (Wye College, University of London) is accompanying the expedition to work in conjunction with the Soils Division of the Department of Agriculture in North Borneo. Mr. Adam Stainton, who has taken part in the botanical exploration of Nepal, organized through the British

Museum (Natural History), is assisting in botanical and photographic work. Other personnel have been drawn from Malaya, Sarawak and North Borneo, with the intention of developing regional interest in the project. The Government of Singapore has made available from the staff of the Singapore Botanic Gardens the services of the botanist, Dr. Chew Wee Lek, and of two plant collectors, Md. Shah and Kadim b. Tassim. Much scientific work on the flora of Borneo has been done at these Gardens, the herbarium of which contains a very valuable set of Bornean plants. Md. Shah is the son of the late Haji Md. Nur, who contributed so much to the scientific work of the Gardens. General vertebrate zoology is in the charge of Prof. J. L. Harrison (Nanyang University), who joined the expedition for a month during June 17–July 22, and Mr. Chin Phui Kong (assistant fisheries officer, North Borneo). Mr. B. E. Smythies (conservator of forests, Sarawak), the author of the recently published book *Birds of Borneo*, is joining the expedition as ornithologist in July. The Forest Department, North Borneo, is assisting in opening up the intended trail up the eastern shoulder of the mountain and across the so-called Pinosuk Plateau, which is of great scenic and biological interest, but apparently of little use for economic development. Various members of the Department are visiting the expedition in its different stages.

The members of the expedition passed through Singapore to Jesselton at the end of May and are spending from June until mid-September on the slopes of the mountain. It is intended to use a portable aluminium sheet hut as a base camp at an altitude of 5,000 ft., whence local expeditions will be made with tents up the unexplored eastern approaches of the mountain. Since exact localization will often be necessary, a set of aerial photographs is being used in conjunction with the War Office and Air Ministry survey maps (1 : 50,000).

Among the many objects of the expedition, special attention is being given to the soil conditions in the mossy forest at 5,000–7,000 ft. altitude and in the areas of ultrabasic rock, to the occurrence of the great parasitic flower *Rafflesia*, to the extraordinary variety of wild figs, and to the opportunity for obtaining photographs to illustrate Mr. Corner's durian theory of tropical forests; Borneo being the centre of the remarkable genus of durian trees.

Local arrangements are being made through the office of the Resident, West Coast Residency, North Borneo, and Messrs. Harrisons and Crosfield have undertaken to act as the Expedition's agents in North Borneo. The botanical and zoological results will be co-ordinated by the staffs of the Royal Botanic Gardens, Kew, and the British Museum (Natural History) respectively.

ATOMIC STRUCTURE OF GRAPHITE AND GRAPHITIZATION

AN informal discussion on graphite, under the auspices of the Faraday Society, was held on January 26 in the Chemical Engineering and Chemical Technology Department of the Imperial College of Science and Technology, London. About 120 people were present, including five from France and one or two others from the Continent.

The programme was opened by the chairman, Prof. A. R. Ubbelohde (Imperial College of Science and Technology), who outlined the scope of the papers to be presented and indicated the kind of problems which it was hoped would be clarified by the papers and subsequent discussion. The first paper, by Prof. C. A. Coulson (Mathematical Institute, University of Oxford), was on the electronic band structure of graphite and the results of a calculation of the energy-levels by conventional tight-binding methods. For the case of a single graphite plane, it is found that the π electrons have energy-levels which fall into two half-bands, just touching one another at the ends where the density of states is zero. Under the influence of neighbouring layers the two half-bands broaden and overlap, so that the valance band is not quite full and the upper (conduction) band is not quite empty. From this energy-level distribution, both the bond-length and the shape of the X-ray emission band can be calculated and they are in good agreement with experimental results. Other properties, such as the electrical conductivity and the de Haas–Van Alphen effect, depend only on the nature of the top occupied levels, and reasonable agreement with the calculation in found in practice. Finally, Prof. Coulson discussed the electronic behaviour at the edges of a graphite layer and the possible situations when either neighbouring sites or a single separated site are involved. Both adsorption experiments and quenching by oxygen are explicable if it is supposed that there are unpaired electrons at a few sites around the edges.

In the following paper, Dr. G. E. Bacon (Atomic Energy Research Establishment, Harwell) reviewed the outcome of the study of graphite by diffraction methods in terms of: (1) the limited crystallographic perfection; (2) the distribution of electrons around the carbon atoms; (3) the texture of artificial graphites; (4) the effect of irradiation by neutrons. The characteristic features of X-ray powder photographs could be explained in detail in terms of random displacements of the carbon planes about the *c*-axis, with a measureable difference in the inter-layer spacing between oriented and mis-oriented planes. The proportion of rhombohedral modification can be varied by mechanical working or by treatment with strong acids or bromine, but the detailed manner in which the transformation takes place is not understood. A combination of neutron diffraction data, careful measurement of X-ray intensities and theoretical calculations has established that the departures from spherical symmetry in the electronic distribution are very small. Irradiation in a nuclear reactor produces significant changes in macroscopic properties, such as elasticity and bulk dimensions, and although these can be explained in general terms as due to interstitial carbon atoms and vacancies, the detailed nature of these defects is still uncertain.

Under the title "Defauts Cristallins dans le Graphite", Prof. J. Mering (Institut National de Recherche Chimique Appliquée, Paris) gave a systematic classification of the various types of defect which can be found in graphite crystals, and discussed their detection and observation and their influence on chemical and physical properties. At the outset

he emphasized the outstanding difficulties in this kind of work—the non-existence of a perfect graphite crystal as a reference standard, and the impossibility of introducing or removing in any simple way faults of a given type. The main classification was into point, line and planar faults. Point faults are the simplest type, but they are difficult to detect by direct means, although they produce relatively simple effects on physical properties (such as electrical resistivity and the Hall coefficient) and on the chemical properties, in particular the tendency to form lamellar compounds. These point faults can be subdivided in terms of substitution atoms, vacancies and interstitials. Faults associated with dislocations and with surfaces are the least simple to define and interpret, but they are detectable more directly by diffraction methods and optical and electron microscopy. Planar faults were subdivided into 'surfaces of dislocations' (such as occur between the not-quite-parallel domains in so-called single crystals of natural graphite or between hexagonal and rhombohedral regions) and errors in layer stacking (such as the orientation faults, which are particularly noticeable in artificial graphites, or random mistakes in an *AB* or *ABC* stacking sequence).

The second session of the meeting dealt with slightly more technological aspects of graphite and was opened with a paper by Mr. P. K. C. Wiggs (Morgan Crucible Co., Ltd., London) on "Elastic Properties of Graphite". Single crystals of graphite have a Young's modulus parallel to the basal planes which is about three times as high as for steel, but parallel to the *c*-axis the modulus is many times smaller. The easiest mode of deformation is, as would be expected, a shear in which neighbouring planes slide over one another. The properties of polycrystalline graphite bear little resemblance to those of the ideal single crystal, and are determined very largely by the disordered material at the junctions between crystallites and by internal voids: such anisotropy as exists is a consequence of the texture which results from the nature of the starting materials and mode of manufacture. As a consequence of this, it is found that all the elastic moduli of polycrystalline graphite are of the same order of magnitude as the low-shear modulus for the sliding of adjacent planes in the single crystal. Only for the more recent developments of pyrolytic graphite and graphite whiskers, where stresses cannot be relieved by sliding, do the high modulus and strength of the single crystal parallel to the planes become apparent. At temperatures greater than 2,000° C., electric furnace graphite is deformed plastically to a very significant extent, but on heating up to this temperature there is a two-fold increase in the elastic moduli on account of the increasing thermal motion of the carbon atoms, which reduces the ability of one plane to slide over another.

A paper by Dr. E. G. Steward (Northampton College, London) described the crystallographic changes which occur during graphitization. There is still considerable doubt about the structural details of poorly crystallized material, which gives very diffuse X-ray diffraction patterns. Recently, there has been a revival of suggestions of non-planar structures, such as a diamond-type of bonding, particularly from electron diffraction observations. As graphitization proceeds and the crystallites grow, the interpretation is much clearer: a progressive modulation of the two-dimensional bands and a general sharpening of the reflexions demonstrates the approach towards the ideal Bernal structure. The ease of graphitization depends on the structure of the crystal nuclei and whether these are separated by cross-linkages. Initial growth occurs by incorporation of non-organized carbon at the crystallite edges, but further progress depends on the existence of near-parallel orientation between neighbouring crystallites. An important characteristic of the final product may be the size of the quasi-perfect macro-units which are much larger than the regions of higher structural perfection revealed by X-ray diffraction. Recent developments in the work on pyrolytic carbons and the examination of moiré fringe patterns in the electron microscope were also discussed.

The final paper, by Prof. W. F. K. Wynne-Jones (King's College, Newcastle upon Tyne), dealt with physical and chemical studies of the graphitization process.

A number of questions and general discussion followed each of the sessions. One of the principal topics was the lack of understanding of the transformation from ordinary to rhombohedral graphite and the energy relations between the two forms. It was also evident that we still have little precise knowledge of the intergranular material which bonds together the individual crystallites, and which is so important in determining the physical and chemical properties of ordinary non-ideal material. G. E. Bacon

RADIOLOGICAL UNITS AND MEASUREMENTS

RADIATION dosimetry is an immensely complicated subject and many of its problems have been only partially resolved. The difficulties are of a fundamental nature and arise: (*a*) from the complexity of the interactions of neutrons and electromagnetic radiations with matter; (*b*) from the still greater complexity of interaction of charged particles, especially electrons, with matter. So long as the understanding of these basic processes remains incomplete, there will be outstanding problems in the dosimetry of ionizing radiations. There is consequently a recurring need—it amounts to a necessity—for authoritative pronouncements on this developing subject to maintain world-wide uniformity in the units, standards and measurements of radiation and radioactivity. The National Bureau of Standards *Handbook 78** is intended to satisfy this need. It supersedes the 1956 report of the International Commission on Radiological Units and Measurements, and the chief interest lies in the difference between the two reports. There has been no major revision and the differences consist almost entirely of accretion and expansion; the new handbook is in fact twice as long as the old. The chief aim according to the preface is ". . . to improve the accuracy of the evaluation of absorbed dose in all places of interest in a patient or other objects". To this end, there is a greatly expanded section on clinical and biological applica-

* United States Department of Commerce: National Bureau of Standards. Handbook No. 78: Report of the International Commission on Radiological Units and Measurements (ICRU), 1959. Pp. x+90. (Washington, D.C.: Government Printing Office, 1961.) 65 cents.

tions, including a new and detailed treatment of energy absorption in and near bone. An important new recommendation for radiotherapists is that the routine recording of bone doses and of tumour doses should now include a statement of maximum and minimum absorbed doses in rads. There is a section entitled "Report on an Inquiry on Current Errors in Clinical Dosimetry" which is somewhat disturbing; it should be carefully studied by all concerned.

In Part 3 ("Physical Aspects of Dosimetry") linear energy transfer and neutron dosimetry are treated in much greater detail, and there are entirely new sections on chemical, solid state, calorimetric and photographic dosimetry. The appendixes contain some interesting and often impressive accounts of international comparisons of various radioactivity standards, and it is noteworthy that Hönigschmid's weighings of radium standards, in which he estimated an error of about 0·02 mgm. (in around 20 mgm.), have now been accurately confirmed by ionization and calorimetric measurements. There are new appendixes on focal spots and the estimation of integral absorbed dose and the final appendix states the International Commission on Radiological Units and Measurements Position Plan adopted at Geneva in September 1958.

Many of the larger criticisms that could be raised against this report are now under active debate in preparation for the major revision referred to in the preface. In a composite production of this nature it is very difficult to preserve consistency. Thus, in referring to the calculated values of mass stopping power ratios (s_m) in Table 8·3, it is stated in footnote 24 "... that an uncertainty of the order of 1 per cent exists in s_m ..." yet later in the same section on page 37 it is recognized that the basis of these calculations is incorrect. The earlier admission on page 1 of errors "... of the order of a *few* per cent ..." would appear to convey the requisite degree of caution. The symbolism is not always consistent and in some places (for example, p. 40 and Figs. 8·6 *b–d*), 'E' is used to denote particle kinetic energy, whereas in other places (for example, p. 51 and Figs. 8·3 *a–c*), 'T' is used for the same purpose.

It is almost superfluous to add that this *Handbook* is essential reading for all radiation physicists, radiotherapists, radiologists and radiobiologists. Unfortunately it is rather unattractively produced, it has several misprints and grammatical errors, and the numerous footnotes are scarcely legible without the aid of magnification.

P. R. J. Burch

THE RED DEER COMMISSION

THE Red Deer Commission was constituted in October 1959 with the general aim of furthering the conservation and control of red deer in Scotland. Its setting up marked the first comprehensive attempt to deal with a controversial problem which has vexed the Highlands for the best part of two centuries.

The introduction of large-scale sheep farming into the Highlands in the latter half of the eighteenth century, replacing the old population of farmers with their primitive breeds of cattle and sheep, resulted, towards the end of that century, in the reduction of deer stocks to probably their lowest level. At the beginning of the nineteenth century another influence was introduced by the development of the sport of stalking, giving a new value to the deer. When prices of wool and mutton declined towards the end of that century, the area given over to deer forests increased rapidly, from less than two million acres in 1883 to more than three and a half million acres at its peak in 1912. In 1952, however, the area of deer forests was estimated to have been reduced to about three million acres.

The influence of these developments on the range and population of deer affected every aspect of highland life, causing deep dissensions. Between 1872 and 1952 six different committees or commissions were appointed by Parliament to make recommendations with regard to conservation and control, but little or nothing was achieved until 1939. The Deer and Ground Game (Scotland) Bill introduced in that year provided powers to control deer, to be exercised by the Department of Agriculture with the assistance of an Advisory Committee and local committees. Developments were prevented by the War, and it was not until 1959 that an Act of Parliament was passed to introduce close seasons for deer in Scotland.

The Act is divided into five parts. Part 1 deals with the setting up of a Commission and its powers. Part 2 specifies close seasons for stags during October 21–June 30 and for hinds during February 16–October 20, to come into force on October 21, 1962. The Commission has now issued its first report*, and, in it, states that, "we consider that if viable stocks of deer are to be maintained throughout the traditional deer forest areas of the Highlands they should not be broken up into isolated fragments. At the same time, if they are not to continue as a serious liability to agriculture and forestry, they must be kept under control. In other words, as grazing animals they should be subject to reasonable standards of management."

The numbers and population densities of deer, unlike those of many other wild animals, do not appear to be self-limiting. In the absence of natural predators such as wolves, or of sufficient killing, deer will increase until they overgraze and damage their habitat, die in large numbers from starvation, or overspill on to agricultural land. Persistent overgrazing must lead to the progressive impoverishment of the hill pastures, on which they depend for their food, and a declining productivity. For the past two centuries, man has provided the only check against this deterioration. The fact that he has not everywhere been exercising sufficient control is the prime reason for the setting up of the Red Deer Commission, with certain over-riding powers, including authority to go on private land in the exercise of its functions.

During the period under review, the Commission has been concerned primarily with the control of marauding deer. Experience this year has shown that it is both widespread and continuous. Where estates are under good management, effective measures are taken to protect tenants and neigh-

* The Red Deer Commission. Report for the period 1st October 1959, to 31st December, 1960. Pp. 22. (London: H.M. Stationery Office, 1961.) 1*s.* 3*d.* net.

bours from damage. There are, however, areas where much more could be done to protect those who suffer. In general, where the laird or factor is permanently resident, satisfactory co-operation and understanding are most easily achieved. Where there is no one of sufficient authority to deal with it on the spot, and the estate concerned is factored from an office outside the Highlands, it is inevitably more difficult.

In some areas where marauding is prevalent there are not enough stalkers and gamekeepers available to deal with the situation. The Commission believes that the widespread marauding taking place throughout the year is probably a symptom of overstocking on recognized deer land in the vicinity, and that the root of all deer 'trouble' is lack of adequate management. The annual kill of hinds, for example, is generally too low; the only way to reduce overlarge stocks is in the culling of hinds. When it is left to an inadequate staff of stalkers or gamekeepers this most important aspect of management is apt to go by default. Sometimes, excessive stocks of deer have been maintained to make allowance for the slaughter done by gangs of poachers. Whatever justification there may have been for this practice in the past, it no longer exists.

Estates in some areas are seriously handicapped in stag shooting by the incursion of the public in the later summer and autumn. If such estates are to be enabled to carry out their obligations during the open (stalking) season, means will have to be found to dissuade the public from disturbing the ground and possibly courting danger to themselves.

WOMEN ENGINEERS IN THE U.S.S.R.

WOMEN form a much higher proportion of engineers and technologists in the U.S.S.R. than in Western Europe or the United States. A study arranged by the Caroline Haslett Memorial Trust was designed to throw light on the factors giving rise to this high proportion, on the capability of women to benefit from a technical education, and on the employment prospects of women so trained. The study included an investigation in the U.S.S.R. itself, and this was undertaken by Miss L. S. Souter, head of the Magnetic Materials Section of the Associated Electrical Industries Research Laboratory, Harlow, and Miss R. Winslade, assistant instruments group manager, Research and Control Instruments, Ltd.

In their report* the authors show that, under the national educational system of the U.S.S.R., co-education of boys and girls is the general practice. In 1943 the sexes were separated into different schools, but, after a ten-year trial, the experiment was abandoned under pressure of public opinion. Only one trace of the separation remains; while boys of 11–14 are having carpentry lessons, girls are being taught domestic science. For all other subjects boys and girls attend the same classes, and, with few exceptions, girls have the same choice of specialities as boys. Comparisons of the performance of men and women as engineers have been made easier by this common educational background. Although the right of entry into university was not fully extended to women until after the 1917 revolution, the limited right of attendance at higher educational courses (some specially set up for women) was granted in 1905 as a result of revolutionary developments at that time and, by 1907, a number of technical institutions were open. There are records of women attending engineering classes at polytechnics during 1907–9, but the numbers were comparatively small.

Equality of opportunity brought the proportion of women in some of the universities up to 50 per cent in 1920. While at the higher medical schools 80–85 per cent of the students were women, the entry into the engineering schools appears to have been much slower. Even as late as 1928, only 1 per cent of the students of thermal power were women; the proportions of women students taking chemistry, physics and mathematics courses was much higher.

The rate of entry of girls into engineering at a lower level appears to have been somewhat faster, and was encouraged by the all-out drive to make good the technical backwardness of the country as a whole. By 1929, 37·1 per cent of pupils at the technicums or trade schools were girls.

In the middle 1930's the entry of girls into engineering institutes increased gradually and then rapidly, partly as a result of the drive to develop industry throughout the country. By 1938 about 28 per cent of the engineering students were women, and by the end of the decade the 30 per cent mark appears to have been passed. Young men were sharing engineering classes with a high proportion of girls and were taught by staffs which in 1939 included 23 per cent women. They came to take it for granted that engineering was not only a possible but also a suitable profession for women.

The increasing proportion of women students in engineering institutes continued after the Second World War, but the rate of increase was falling; the numbers are now increasing slowly, and there has been negligible change in the 33–35 per cent level reached in the late 1940's. There has, however, been a considerable increase in the number of women holding higher engineering qualifications. The industries which attract women engineers show marked differences from Great Britain. Here, industries with a long tradition of women operatives are slow to attract (or accept) women engineers; in the U.S.S.R. it is the textile and food industries which have the highest proportion of women engineers. The high proportion of women engineers in the U.S.S.R. was so much taken for granted that the reasons for the Souter–Winslade study were frequently called into question and led to no little amusement.

Throughout the U.S.S.R. women are forbidden to take up any branch of engineering which is considered to be prejudicial to health. Some examples are: (1) at trade-level—girls are not permitted to service television sets because of the danger of radiation and because of the heavy weights of the sets themselves; they are permitted to train as telephone service engineers, but not allowed to carry out work involving the climbing of roofs or poles, or to work

* Caroline Haslett Memorial Trust. *Women Engineers in the U.S.S.R.: a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R.*, carried out under the auspices of the Caroline Haslett Memorial Trust. By L. S. Souter and R. Winslade. Pp. 28. (London: The Caroline Haslett Memorial Trust, 25 Foubert's Place, W.1, 1960).

on underground cables; (2) at professional level—girls are permitted to study as mining engineers and may exercise their profession in surface jobs, but they are forbidden to work underground.

Strong attempts are made to discourage girls from some types of work which are regarded as unsuitable although not forbidden by law. These posts fall into the categories of physically dangerous or arduous, such as forging or rolling processes, or unpleasant, such as some of the processes of chemical engineering. As a general rule, there are fewer women in heavy industry than in the textiles, food, watch and clock industries.

The Krasny Oktyabr sweet factory appears to be typical of the food industry, and here a women occupies the post of director, and women are in charge of the departments concerned with planning (engineering economist), costing (economist), analytical planning, technical control of output, mechanical engineering and design. The women acting in a supervisory capacity are: the deputy chief mechanical engineer, 15–20 design engineers and 2–4 production shop supervisors. Women head most of the ancillary departments, including book-keeping, library, polyclinic and canteen. Men occupy the posts of commercial director (combined with the post of vice-director), chief engineer, chief mechanical engineer, head of wages department and 2–4 of the production shop supervisors. There was general agreement that girls and women show a particular preference for engineering economics, engineering design, laboratory work and teaching. Engineering economics, in particular, is popular with girls, and, until the current year, made up 95 per cent of the students of faculty in the Leningrad Polytechnic. Engineering economics includes the economy of distribution and supply of energy, metallurgical production and processing, mechanical engineering, machine construction and mechanization of working processes by automation.

Preference for engineering design work was illustrated in the teaching institutes by the number of women students in courses on airfield construction, road, bridge and tunnel design at the Institute of Ways of Communication; the figure of 35 per cent compared with 10 per cent in the mechanical faculties.

The attraction of research work was evident, and the proportion of women in all types of laboratories, such as those concerned with fundamental research, development, works-aid, and material or process control, was much higher than the 28 per cent average. Almost all the research laboratories of the Likhachiov Motor Works are headed by women and some completely staffed by women. 50 per cent of the staff of the Research Institute of Common Technologies are women, and here women head a fair proportion of the sections. 60 per cent of the staff of the Research Institute for Communications (covering telecommunications) are women.

There is a general shortage of teachers, and there is a drive to make more junior teaching posts more attractive. Engineers are released from industry to the teaching institutions on a part-time basis; no extra hours per week are worked and extra pay is given. Nevertheless, it is difficult to attract people to teaching. In spite of the fact that teaching is said to be fairly high in order of popularity with women, and most teachers of mathematics and physics are women, they comprise only about 20 per cent of the teaching staffs of these institutions, that is, less than the national average among engineers.

Men and women receive equal pay, and there is no reason why women employees should be preferred on this score. The minimum age for retirement for women is 55 compared with 60 for men, but both men and women often continue working for up to ten years after the minimum age.

It was not possible to make a comparable study of the distribution of girls between various trades which are taught in the technicums or of the girls obtaining technical qualifications at junior technical colleges. The authors considered the question of whether or not women in the U.S.S.R. are allowed and encouraged to enter the engineering professions and also whether they achieve promotion to top positions.

In 1957 women formed 10 per cent of the directors, chief engineers and other leading specialists in the national economy, as opposed to 38 per cent employed as engineers. The total figures for all ranks of engineers show that 28 per cent of the positions are occupied by women.

In the U.S.S.R. Miss Souter and Miss Winslade also examined the objections put up to exclude women from senior positions in engineering in Great Britain. They found that girls were putting up performances in mathematics and physics comparable with boys; although the proportion of prizes going to girls varied from year to year; over a ten-year period they averaged a share of the prizes in relation to the total numbers. There is a slight departure from the general trend in Kharkov where, in the Institute's entrance examination, girls put up a slightly better performance than boys in physics, chemistry and foreign languages, while boys do better in mathematics and Russian. At all stages the adequacy of the mental capacity of women was so taken for granted that some difficulty was experienced in bringing discussion with scientists in the U.S.S.R. to a factual level. Schoolgirls appear to have no inhibitions about tackling technical work, and 40–45 per cent of the technical streams of secondary schools are girls. These girls show much enthusiasm for all types of technical work and, in some schools, have the reputation of being more accurate with machine tools than boys.

THE DUST CLOUD ABOUT THE EARTH

IN *Nature*, 189, 127 (1961), Dr. F. L. Whipple discusses four possible explanations for the concentration of dust near the Earth. Of these the most favourable consideration is given to the hypothesis that it originates in collisions between meteorites and the Moon.

I would like to propose as at least equally tenable a fifth hypothesis: that the dust originates from meteorites that graze the Earth's atmosphere. Clearly meteorites that strike the Earth's atmosphere at too large an angle (or with too small an impact parameter) will be burnt up in the conventional way. Clearly,

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too, meteorites that approach the Earth with too large an impact parameter will not be slowed enough to have their orbits greatly changed. Between these limits, however, must be some the velocity of which, on re-emergence from the atmosphere, has been reduced to within the range of 7–11 km./sec. which will lead to their capture by the Earth's gravitational field. Those larger and faster will of course be broken up, liquefied or even vaporized in the process. Particles with an initial speed of 15–20 km./sec. and of masses up to perhaps 10^{-5} gm., however, might be expected to radiate well enough to dissipate their excess energy without complete disintegration. Even if the impact parameter for any one particle must lie between limits only 1 km. apart, perhaps in the region of 100 km. above the Earth's surface, the cross-section available for interplanetary material will be nearly one-tenth the total cross-section of the Moon. All this material will have been captured by the Earth's gravitational field, whereas the fraction of material splashed off the Moon to be so captured must be exceedingly minute.

Of course, most of the captured material will be lost in the atmosphere at the very next perigee of its new orbit. The Moon is still required for this hypothesis although not as an actual source of supply. Its importance is that it will distort the paths of those particles which have long elliptical orbits approaching it at all closely; roughly half these distortions will make atmospheric capture even quicker by reducing the particle's energy, but the other half will give a gain of energy which will increase the radius of approach to the Earth at perigee and, in some cases, allow the production of the very long-lived orbits required.

The range of initial orbits, from which such quasi-permanent capture is possible, is difficult to estimate, but it seems to me to be at the very least as probable a process as that described by Dr. Whipple.

Consideration of the method of slowing down and of capture suggests the possibility that a higher proportion of iron–nickel particles might be found in the local than in the interplanetary dust and that there might be a preponderance of particles revolving about the Earth in the same sense as does the Moon. The former, though not the latter, suggestion would be reversed according to the lunar-collision hypothesis.

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Dr. Whipple[1] has given an excellent summary of rocket, satellite and space-probe measurements demonstrating the existence of a high concentration of interplanetary dust near the Earth. It is the purpose of this article (1) to show in more detail than presented elsewhere[2] how this dust blanket may reasonably be expected to arise, (2) to correct the unfortunate although quite justifiable misinterpretation Whipple[1] has made of my theory[2], which was described too concisely, and (3) to show that Whipple's[1] different spatial dependence of the observations are not in disagreement with the theory presented here in greater detail.

Wyatt and Whipple[3] have shown that the most minute cometary debris acquires nearly circular orbits due to the relativistic aberration of solar radiation pressure[4], and that the debris spirals into the Sun with a radial velocity of twenty to six hundred Earth radii a year at the position of the Earth's orbit, depending on the effect of solar corpuscular radiation[5]. By solving the Lagrangian equations of motion of the particles[2] in the combined solar and terrestrial gravitational fields, one may determine the orbits which the particles follow as they approach the planets during their inward motion. It is important to note that, independent of angular momentum considerations, the planets will attract and concentrate the dust in the ecliptic plane as is readily determined by solving the equation of motion in a direction perpendicular to the ecliptic plane. As discussed in a previous paper[2], inclusion of this effect will cause the concentration of dust in the plane of the ecliptic to vary as the inverse three-halves power of the solar distance which is observed[2] rather than the inverse first power which would be predicted in the absence of the planets[2].

The results for motion within the ecliptic plane may be much more simply understood, however, if one examines their motion in an Earth-centred co-ordinate system and (except for the relative earth-particle velocity due to their differing solar distances) neglects the motion of the Earth and particles about the Sun. This neglect may be shown to be inconsequential when one transforms the exact Lagrangian to an Earth-centred co-ordinate system. Under these conditions the particles encounter the Earth in an initially hyperbolic orbit and a negligible quantity of them enters captured orbits even temporarily. The theory assumes only hyperbolic orbits about the Earth. Even those few which are captured are soon lost due to the effects of radiation pressure, which is a major force on such slight objects as to be affected by the Poynting–Robertson radiation effect[3,4].

Whether or not the particles encounter the Earth (that is, impinge on the atmosphere and are present at low satellite altitudes of a few thousand kilometres) is determined by the conservation of their angular momentum. At infinity, their velocity with respect to the Earth is at least that due to their different solar distance, r:

$$v_\infty = \sqrt{\frac{GM_\odot}{r}} - \sqrt{\frac{GM_\odot}{D}} \sim \frac{1}{2}\sqrt{\frac{GM_\odot}{D}}\,\frac{a}{D} \qquad (1)$$

where $a = r - D$, D is the radius of the Earth's orbit, G the universal gravitational constant, and $M_\odot$ the mass of the Sun. If they are in a circular orbit about the Sun, their moment arm with respect to the Earth is initially $\sqrt{(a^2 + h^2)}$ where h is the perpendicular distance of the particles from the ecliptic plane. (Since the dust is mainly concentrated in the ecliptic plane, most of it has negligible inclination to the ecliptic.) The velocity of the particles at perigee is due to their fall in the Earth's gravitational potential, if one assumes their initial velocity to be slight. Hence in order to reach a distance, b, from the Earth's surface, a and h must satisfy the condition:

$$\frac{1}{2}\sqrt{\frac{GM_\odot}{D}}\,\frac{a}{D}\sqrt{(a^2 + h^2)} \leqslant b\sqrt{2\frac{GM_\oplus}{b}} \qquad (2)$$

in order for the particle's angular momentum about the Earth to be conserved. $M_\oplus$ is the mass of the Earth. Hence, in order to reach the surface of the Earth:

$$a\sqrt{(a^2 + h^2)} \leqslant (2D)^{3/2}\sqrt{M_\oplus/M_\odot} = 1{\cdot}76 \times 10^4 \quad (2a)$$

where distances have been expressed in terms of the radius of the Earth. Fig. 1 illustrates the maximum

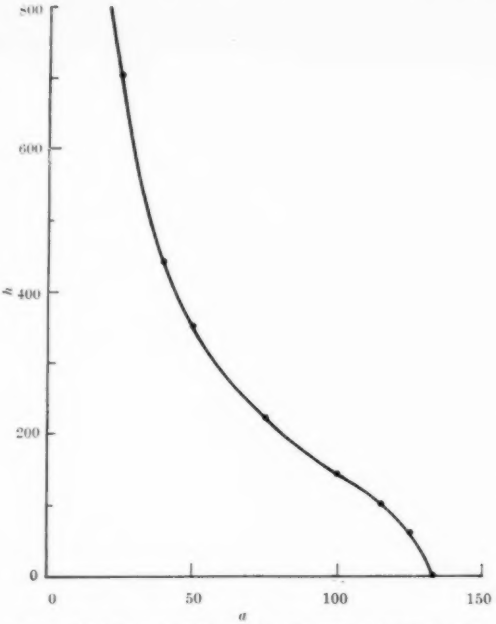

Fig. 1. Maximum permissible solar distance, r, different from the Earth's orbital radius, D, for interplanetary dust initially in a circular orbit about the Sun to reach the Earth's surface. The abscissa, $a = r - D$, and ordinate, h, the distance from the plane of the Earth's orbit, are expressed in terms of the Earth's radius. Values of h and a to the right of the curve require the incident dust to have too much angular momentum to reach the surface of the Earth ($a = 1$)

distance from the Earth in the plane perpendicular to the Earth's orbit from which circularly orbiting dust may be captured. The effect of radiation pressure would be to shift the curve to larger positive values of a, but much the same effective capture cross-section would result. All particles within a radius where their terrestrial gravitational potential exceeds their kinetic energy with respect to the Earth will have their orbits materially affected by the Earth's pull, but they will not be captured (that is, encounter the Earth's atmosphere) unless equations 2 and $2a$ are satisfied.

The radial velocity of the dust (with respect to the Earth) as a function of distance from the centre of the Earth, l, may be found by assuming a particle's total kinetic energy is due to its fall in the Earth's field and its angular momentum $\boldsymbol{l} \times \boldsymbol{v}$ is a constant equal to the left-hand side of equation 2.

$$v_l = \sqrt{2\frac{GM_\oplus}{l}(1 - b/l)} \sim \sqrt{2GM_\oplus/l} \qquad (3)$$

is the equation for the radial velocity of a particle in a hyperbolic orbit, where b is the perigee distance.

The total flux of dust entering a sphere of radius l must be a constant given by:

$$4\pi l^2 v_l n_l = \text{constant} \qquad (4)$$

If the angular dependence is neglected, one obtains an average density for dust approximately equal to:

$$n_l \sim n_0(l_0/l)^{3/2} \qquad (5)$$

where l_0 is the effective range of the Earth's gravitational field, and n_0 is the particle density in the absence of planetary condensation. For $l \sim b$, n_l would vary as l^{-2} or faster. This effect is compensated in part by those particles within the Earth's gravitational pull the initial positions of which exceed the condition expressed in equation 2 for b equal to the radius of the Earth. Those particles populate the region next to a planet, but are not captured.

Excellent observations of dust-scattered light in the solar corona show that the concentration of dust in interplanetary space[2] is 10^{-14}–10^{-15} particles per c.c. at the position of the Earth's orbit. Yet space-vehicle observations[1] give flux densities of 10^{-12} particles/cm.^{2} at a height of 3,000 km. The dust most probably has a velocity of only $\sim$ 11 km./sec.[4]. Thus, these observations provide the best available evidence for planetary condensation of dust forming a blanket about the planets. Since n_0 is most likely 10^{-15}/c.c., l_0 would have to be about 100 Earth radii.

Rocket observations at about 100 km. give a higher concentration of dust still consistent with the largest estimates of the theory presented here, but indicating a more rapid decrease with height than $l^{-3/2}$ (ref. 1). A more rapid increase in dust concentration with decreasing height than the $l^{-3/2}$ prediction is to be expected from equations 3 and 4 for l approximately equal to perigee, b. Thus if much of the dust had a perigee close to one Earth radius, $n_l \sim (l - b)^{-1/2}$ would be more in agreement with the observations. Furthermore, space-vehicle measurements are of momentum, not mass, and detector thresholds might be expected to decrease rapidly with height in some cases, because particle gravitational energy decreases with height ; since particle number decreases rapidly with particle mass, detectors would count smaller, more numerous particles at lower altitudes in those cases when the relative velocity of particle and vehicle depends appreciably on height. De Jager and Hoffmeister[7] have written interesting, although quite different accounts, of the same subject.

I am pleased to thank Dr. F. S. Johnson for his interest in and support of this work and Dr. F. L. Whipple for pointing out the necessity for a more complete discussion of the theory.

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Dr. Fremlin's suggestion of a contribution to the dust cloud near the Earth by meteorites that graze the Earth's atmosphere cannot be disproved. On the other hand, estimates of the total contribution to the Earth by meteorites, for example, G. S. Hawkins[1], add up to only a few tons a day in orbits hyperbolic with respect to the Earth. The total input[2], however, appears to add up to hundreds or even possibly thousands of tons a day. Hence, I should expect the fractional contribution by meteorites to be minor.

In Dr. Beard's preceding article I am pleased to see my statement of his point of view corrected. His conclusion, however, is subject to question. Though the dust particles producing the zodiacal light may well move in nearly circular orbits about

the Sun this fact is not yet demonstrated. That their orbital inclinations to the ecliptic are negligible is really very doubtful, unless collisions among them can be proved to reduce the inclinations markedly. Haug[3], for example, finds that the light in the zodiacal cloud is fairly well represented by the distribution of orbital inclinations found for photographic meteors. Thus, the mean velocities of the particles with respect to the Earth and hence the effective gravitational cross-section of the Earth for the particles may not be much greater than its geometrical cross-section, rendering Beard's process ineffective.

From acoustical impact measurements on the satellite *Explorer I*, Hibbs[4] finds, in contrast to the statement in my earlier article, but in agreement with my suggestion, that the evidence strongly indicates that the particles are moving in orbits about the Earth. I am glad of the opportunity to make this correction to the earlier article and to note that particles ejected from the Moon may move in all types of orbits about the Earth. Thus, my predicted concentration towards the Moon's orbit in direct motion may easily be wrong, depending sensitively on the velocity distribution of particles ejected in lunar impact explosions.

Singer[5] finds that particles passing by the Earth isotropically in hyperbolic orbits should demonstrate a shadowing effect of the Earth such that impact-rates on surfaces very close to the Earth's surface will be somewhat smaller than at a distance of a few thousand kilometres. The observational data concerning the number of meteoritic dust impacts made by rockets are highly uncertain and may be much over-estimated. Thus the increase of concentration very near the Earth may well be smaller than I concluded in my earlier article, but still very high. It is clear that more observational data and more detailed theory are needed to clarify these matters.

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ANALYSIS OF POLARIZED LIGHT BY THE BEE'S EYE

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AND

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BEES, like some other arthropods, can orient various movements according to the plane of polarization of the incident light[1]. Such performances have been explained widely on the basis that the compound eye contains an analyser for polarized light. Another view has been proposed, namely, that environmental clues (for example, reflectance patterns) which depend on plane of polarization are perceived instead[2,3].

If the bee's eye is in fact an analyser for polarized light, the analysis could occur either in the dioptric apparatus of the eye or in the receptors (rhabdomeres) themselves. All efforts to locate the analyser in the dioptric apparatus by examining cornea and crystalline cone for dichroism have, however, met with failure. Because of this, and since disagreement exists about the behavioural evidence for or against extra-ocular analysis, it is thought that the receptors themselves are polarization analysers.

The method by which this is most often thought to occur was originally described, as a hypothetical model, by Autrum, and adopted by von Frisch[1] to explain bee orientation to polarized light. Later it was given some physiological substance by Autrum and Stumpf[4]. Briefly, the theory proposes that the opposite pairs of retinula cells with their rhabdomeres, which are shaped in ommatidial cross-section like the segments of an orange, have specific 'sensitive planes' of polarization, owing to their structure or to the orientation of pigment molecules within them (Fig. 1). The latter explanation, in particular, is favoured by Stockhammer[5]. Some support for the notion that the rhabdomeres can act as analysers has come from the demonstration that fixed rhabdomeres are doubly refracting[6,7]; they are, however, more weakly so when fresh, and are not dichroic in any circumstances[8]. The mere existence of molecular orientation would in any event scarcely prove that the structure is used in polarization analysis. With the arrangement of rhabdomeres shown in Fig. 1, in which the arrows in the individual rhabdomeres indicate the 'sensitive planes' of polarization, a polarized light stimulus will always stimulate the same number of receptors regardless of its plane. Thus the electrical output of the ommatidium or of a population of ommatidia should be independent of the plane of polarization. Autrum and Stumpf have shown that this is true for the bee's eye. De Vries *et al.*[9], however, were able to find no 'ripple' in the electro-retinogram from single ommatidia or the whole eye in *Calliphora*, when the plane of polarization was rotated at various frequencies.

A somewhat different sort of electro-physiological test has been applied to this problem more recently. It has yielded results which, we believe, are inconsistent with the Autrum model, like those of de Vries *et al.* The experiments to be described were actually done in 1958 and communicated in the form of abstracts[10]. We report them now in detail because the Autrum model is so widely invoked to explain the orientation of polarized light[5], and even as an aid in interpreting the fine structure of insect rhabdomeres[11]. Von Frisch *et al.*[12], in a recent review, have treated our preliminary note in a surprising manner. They describe it, quite correctly, as being without methodological detail, and then reject its conclusions by citing a personal communication from

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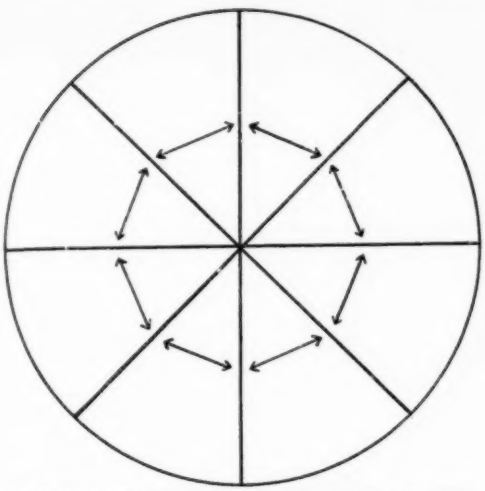

Fig. 1. The model of the bee's ommatidium proposed by Autrum and Stumpf (ref. 4). The arrow located in each rhabdomere indicates its 'sensitive plane' of polarization; opposite pairs of receptors are identical in this respect

Autrum, who suspects that we had not taken certain sources of error into account. Although this is possible, it is difficult to know how Prof. Autrum could have a reasonable basis for such a technical criticism, since no experimental detail was supplied in our abstract. Nevertheless, von Frisch *et al.*[12] had sufficient confidence in this opinion to state, in their summary: "Electrophysiological, polarization-optical and electron-microscopical studies have indicated unanimously that polarized light is analysed within the compound eyes of insects, in the rhabdomeres of the visual cells".

The technique used in our experiments was that of selective adaptation, a method which has been applied with success to other problems in sensory physiology in which the aim is to separate sub-modalities from a compound response. The retinal action potential or electro-retinogram of the honey-bee's eye was recorded from the isolated head preparation with sharpened steel needle electrodes, amplified with a capacitance-coupled pre-amplifier (time-constant of 1 sec., sufficiently long not to distort the responses to brief flashes) and displayed on a cathode-ray oscilloscope from which photographic records were made. The adapting and the stimulating light occupied nearly coincident optical axes, approximately normal to the corneal surface at its centre. The adapting source was a 300-W. tungsten projection bulb, the stimulating source a 20-W. microscope lamp. Stimuli were delivered through a photographic shutter, and more than covered the corneal surface. In the normal experimental procedure, a test-flash was delivered to the dark-adapted eye through a 'Polaroid' filter. The eye was then adapted to light for 30 sec. with the 'Polaroid' in the same position; after this, the course of dark-adaptation was followed by exposing the eye to test-flashes of the same intensity, duration and polarization plane as that which preceded the adaptation. After dark-adaptation was complete, the experiment was repeated with only one difference: the 'Polaroid' filter was rotated 90° for the adaptation period only. The first procedure will be referred to as a 'control' and the second as an 'experimental' adaptation. In effect, then, the procedure tested whether adaptation in a particular plane of polarization spares any significant proportion of the population of receptor units. According to the Autrum hypothesis, those rhabdomeres having 'sensitive planes' different from the planes of polarization of the adapting light should be less affected by it. Therefore, one would expect less light-adaptation, and/or faster recovery in the dark, when the planes of polarization of adapting light and test flash differ.

The intensity of all test-flashes used in these experiments was 125 ft.-candles; that of the adapting light was 3,200 ft.-candles. Test-flash durations were usually 0·1 sec. The intensity of the test-flashes was chosen so as to be in the region where, for most preparations, the change in electro-retinogram amplitude is large for a small intensity increment. Electro-retinogram amplitudes were measured on responses photographed on moving paper from the oscilloscope trace. In all experiments, test-flashes were delivered 7, 12, 17, 22, 32 and 42 sec. following the end of light-adaptation. Dark-adaptation was virtually complete within 1 min., in agreement with the observations originally made by Autrum[13] that the eyes of certain insects (including the bee) are 'fast' in terms of adaptation-rate and the ability to follow flickering stimuli. In plotting the time-course of dark-adaptation, successive amplitudes are expressed as a percentage of the amplitude of the response to the initial flash, before adaptation. In most, adaptation is not quite complete at 42 sec.; but two series, in which the response amplitude at 42 sec. was less than 80 per cent of the dark-adapted value and in which, therefore, some overall change in sensitivity had clearly occurred, were rejected.

Table 1 shows the results of experiments on the extent of light-adaptation produced by the two procedures described here. The figures given are amplitude of the electro-retinogram taken 7 sec. after the end of light-adaptation and expressed as percentages of the initial dark-adapted value. The experiments have been done in pairs, the control either immediately preceding or following the experimental one in the adjacent column. In all pairs of experiments, the difference between adaptation in the same plane of polarization or the perpendicular one is small. The paired experiments naturally differ from one another, since the relationship between the test-flash and adapting intensities used and the sensitivity of the electro-retinogram may vary from preparation to preparation. The important aspect of the results is the high degree of internal consistency shown by the paired experiments. The average reduction for the two groups is identical.

Fig. 2 shows complete dark-adaptation curves, averaged from data such as those shown in Table 1. Six control and six experimental runs made on four

Table 1. Extent of Light-Adaptation, tested by Stimuli in the same Plane of Polarization as that of the Adapting Light (Control) and in the Opposite Plane

Amplitude of responses recorded 7 sec. following the light-adaptation, and expressed in per cent of the amplitude before adaptation

Preparation	Experiment No.	Control	Experimental
2	1	79	67
2	2	58	62
3	1	80	80
3	2	77	77
4	1	74	82
4	2	72	64
5	1	69	66
	Average	73	73

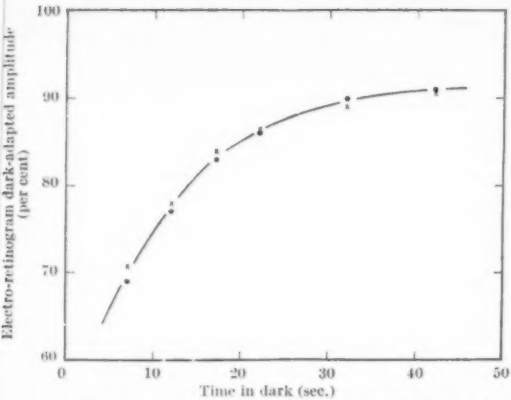

Fig. 2. Dark-adaptation curves for the bee's eye. Each point is the average of six runs on four preparations. 'Control' adaptations are indicated by crosses, 'experimental' adaptations by filled circles

different preparations have been averaged. The two average curves are virtually identical.

These results show that there is no population of receptors contributing to the electro-retinogram which can be adapted selectively to a measurable extent by exposure to intense plane-polarized light. It may be noted that 'contribute' in the sense used above does not mean 'generate'; the receptor cells may or may not play a large part in generating the electro-retinogram, but they surely trigger activity in those post-synaptic units which do generate it directly. For our purposes, the distinction is meaningless, because the effects of adaptation are felt at all levels, not just at the primary one. The electro-retinogram is used only because it is a convenient test of the sensitivity of a system.

The results seem to us inconsistent with the Autrum hypothesis. Since our experiments were performed, however, new evidence in support of the hypothesis has been produced. Some investigators[14,15] have recently obtained intracellular records from two dipteran eyes, *Lucilia* and *Calliphora* respectively. Both groups assumed that the responses, 'generator' potentials without spikes, originate from retinula cells; both show that, in some units at least, the amplitude of the generator potential depends on the plane of polarization of the stimulating light. In the Burkhardt and Wendler experiments, the difference between the response produced by the most effective and least effective planes of stimulus polarization was equivalent to a 46 per cent difference of intensity.

There is no question that these results provide strong support for the Autrum model. In attempting to resolve our own failure to obtain, by selective adaptation, results consistent with the model, three possibilities exist: (1) the inconsistency is explained by a real difference in property between the bee's eye and the dipteran eyes investigated; (2) our experiments lacked the sensitivity required to reveal a differential polarization sensitivity; or (3) the results on recording from single retinula cells do not represent a real act of physiological polarization analysis by the sensory cell. Possibility (1) cannot be evaluated at this point, but does not seem likely. We cannot rule out possibility (2); but it would be surprising if a peripheral mechanism producing, under the favourable conditions of our experiment, a differential polarization sensitivity too small for us to detect, could be useful under natural conditions, where the bee must analyse rather weakly polarized light from the sky. If each retinula cell in the bee's eye actually responded to its 'sensitive plane' of polarization as though it were twice as bright as that 90° away, a selective adaptation experiment of the kind we performed would surely have revealed sensitivity differences; furthermore, steady illumination in one plane of polarization abruptly changed to a plane differing by 90° would be expected to recruit an additional response from less-adapted elements. We have not been able to show such 'shift' responses in the electro-retinogram in our own experiments, nor could de Vries *et al.*[9] with somewhat different methods in *Calliphora*.

The third possibility, that the results from intracellular recording do not indicate real polarization analysis by the sense cell, must at least be considered. In the first place, the responses in question are only inferred to come from retinula cells; there is no way, at present, of being sure that they do. Since such cells are certainly the most likely source of the records, however, it seems fair to assume that they do. Burkhardt and Wendler show records from a single cell, and state that the difference in amplitude of the potential produced by light stimuli having polarization planes 90° apart is equivalent to a 46 per cent difference of intensity. They say that a "large number" of penetrated cells showed such differences, but do not specify what the number is, what percentage of the total observations it represents, or what is the average amplitude difference for different stimulus planes of polarization. Even if one concludes that, in all cells showing the phenomenon, it had the same magnitude as the illustrated case, one has no idea how many cells failed to show it at all. The authors state, indeed, that some did not; and in fact their conclusion that the property of polarization analysis may be restricted to certain retinula cells already involves a departure from the Autrum model in its original form. Those cells in the Burkhardt and Wendler experiments which did show differential responses to different planes of polarization exhibit one rather peculiar property: the difference is dependent on wave-length, appearing only with short-wave-length stimuli. If orientation of the photo-sensitive pigment within the ultrastructure of the rhabdomere is the critical factor in polarization analysis, as proposed by Stockhammer[7], it is difficult to understand why the vibration planes of blue light would be critical in determining absorption and the vibration planes of red light unimportant.

It is well known that structures optically interposed between a source of polarized light and a detector can create a kind of polarization sensitivity by differentially refracting, reflecting or absorbing light of particular *e* vectors. It is possible, though by no means certain, that the intracellular micro-pipette introduced into the retinula cell could act in just this way; if it did, then the results could be explained as an act of polarization analysis by the recording micro-electrode. The absence of the effect in some cells, and its presence in others, might thus be related to the depth of penetration, or to the orientation of the micro-electrode with respect to the stimulating light. Electrodes with tip diameters of less than 0·5μ, the kind used for these experiments, are able to record extracellular potentials only very locally. It may be that a solution to the problem raised by the possible optical action of the penetrating micro-electrode might be to record outside the cells. In

some circumstances at least, the micro-electrode should record dominantly the output of one retinula cell, and if the latter indeed varies with stimulus polarization, the recorded extracellular potential should vary too. Burkhardt and Wendler refer briefly to extracellular recordings in connexion with the experiments and state that their amplitude did not change with plane of polarization. It would be an exceptional circumstance if an extracellular micro-pipette of the diameter used recorded equally from all members of a given ommatidial cluster of retinula cells.

At this point, it is evidently impossible to decide which of the three possibilities is correct. The Autrum model continues to be the most attractive explanation of polarized-light sensitivity in insects. Our results are, however, not consistent with it; and it seems reasonable to ask that before it is given the kind of total acceptance accorded it by von Frisch *et al.*[12] and other behavioural workers, direct and unambiguous evidence be brought in its favour.

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ENHANCEMENT OF INHIBITION OF INTESTINAL ABSORPTION BY CETRIMIDE–PHLORIDZIN COMBINATION

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THE inhibitory effects of trimethylhexadecylammonium bromide (cetrimide) and stearate on the intestinal absorption of glucose in the rabbit, and of glucose, sodium butyrate and methionine in the rat were described by me earlier[1,2]. Later, the anæsthetized mouse was used as a test preparation for the study of intestinal absorption as well as for the rapid assay of substances capable of influencing this function. In this animal, cetrimide was found to exhibit its greatest inhibitory effect on the absorption of glucose, while sodium butyrate and methionine absorption were inhibited to a moderate extent. Phloridzin, on the other hand, which was also found to inhibit the absorption of glucose markedly, and that of butyrate moderately, had no effect whatever on the absorption of methionine. Differences shown by these and other species in the actions and activities of cetrimide and of phloridzin suggested that the effects were enzymic in nature, but further elucidation of the site and mode of action of these substances was desirable. It was considered that the effects of simultaneous intestinal perfusion of these two compounds might throw additional light on this problem, particularly with regard to such matter as synergism, potentiation and antagonism, as well as effect on phosphatase activity. A preliminary report of the results of this investigation is given in this article, while a more detailed account will be published elsewhere.

The effects of cetrimide, phloridzin and combinations of the two substances on the intestinal absorption of glucose, sodium butyrate and methionine were compared by means of the mouse assay method, using both the half-hour and the five-minute intestinal perfusion techniques, which will be described elsewhere. Pure interbred mice of C_3H and *CBA* strains were used. Cetrimide and phloridzin were perfused in concentrations of 0·001, 0·002 and 0·01 per cent singly and in combination, on groups of four animals each, while the concentration of three nutrients investigated was always 0·2 per cent. Chemical estimation of the three substances in the perfusate at the end of the experiment was carried out and their percentage absorption was calculated.

Striking enhancement of the inhibition of the intestinal absorption of both glucose and of butyrate was obtained when equal concentrations of cetrimide and phloridzin were perfused simultaneously. This effect was most noticeable in the case of glucose absorption in the half-hour perfusion experiments, and the results are given in detail in Table 1. In the range of concentrations used, the regression of response on the logarithm of the drug concentration was linear in all three cases (Fig. 1) for half-hour experiment and highly significant, while deviations from regression were not significant.

Table 1. PERCENTAGE ABSORPTION OF GLUCOSE, IN EACH OF FOUR MICE, FROM INTESTINAL PERFUSION FLUID CONTAINING DIFFERENT CONCENTRATIONS OF CETRIMIDE, PHLORIDZIN OR CETRIMIDE + PHLORIDZIN

The fluid was perfused for half an hour in each experiment

Concentration of drug (per cent)	Cetrimide	Phloridzin	Cetrimide + phloridzin
0·001	44·1	18·8	10·2
	40·6	35·7	11·1
	51·9	32·4	3·9
	49·9	39·5	2·7
	Mean 46·625	31·6	6·975
0·002	23·3	19·2	9·4
	27·0	19·6	5·8
	41·3	16·8	2·8
	29·2	17·9	2·6
	Mean 30·02	18·375	5·15
0·01	16·8	10·2	−3·6
	36·5	1·7	−4·4
	28·4	7·9	1·7
	14·6	10·5	−0·2
	Mean 24·075	7·575	−1·625

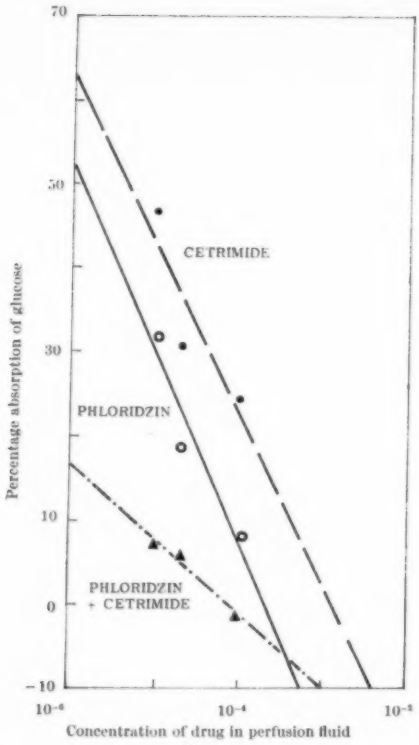

Fig. 1.

The slopes of the dose-response lines were practically the same for the two drugs when these were used singly. When the drugs were used in combination, however, the slope was significantly different. The main statistical results together with a skeleton analysis of variance appear in Table 2.

Phloridzin was about five times as potent in inhibiting the intestinal absorption of glucose in the mouse as cetrimide, while the two substances perfused together were about ten times as active as phloridzin alone. Since the slope of the cetrimide + phloridzin dose-response line differed from that of phloridzin or of cetrimide alone, the potency ratio assumes different values at different concentration-levels, and would be given by the antilogarithm of the distance between any two points taken parallel to the concentration co-ordinate. The value 10·45 of this ratio given in Table 2 was calculated on the assumption of parallelism, and would represent an average. The concentration of cetrimide which produces this enhancement was only 1/10 × 1/5 or 1/50 of that which would be required of this substance to produce the same inhibition of glucose absorption when used alone.

The combination of the two drugs also gave enhanced-inhibition of butyrate absorption. Though the butyrate absorption figures were considerably scattered, so that precise determination of the fiducial limits of the potency ratio was not possible, the potency ratio value of twelve was of the same order as that obtained in the case of glucose. No enhancement of the inhibitory effect on methionine absorption was obtained, however.

The five-minute assays gave an enhancement value of six for the inhibition of glucose absorption. The lower figure obtained in these experiments was the result of the relatively long time required by cetrimide before it exhibits its full effect.

The inhibition of methionine absorption was unchanged, though the inhibition of glucose and of butyrate absorption became much more pronounced when the two drugs were combined. Hence, it seems more likely that the basis of this increase in effect is an enhancement of the action of phloridzin by cetrimide rather than the opposite. This enhancement occurred at a considerable range of concentrations, and its magnitude is probably among the greatest known in pharmacology.

The synergism shown between cetrimide and phloridzin in the inhibition of intestinal absorption is not simply additive. From this it may be concluded that when the drugs act independently, their effects are exerted at different sites in the absorptive mechanism. The degree of enhancement in the combined action is also greater than can be explained by assuming two different sites of inhibition in the same biochemical chain of reaction, though this may in fact represent their basic mode of action. A third mechanism has to be invoked to explain the magnitude of the effect. The formation of a cetrimide–phloridzin complex, which is more active than either compound alone, though theoretically possible, does not seem probable. A conserving action of the cetrimide on the phloridzin appears more likely. This may be due to the protection of the latter drug by the former from rapid destruction or clearance, but the fact that there is no fall in the activity of either compound in half-hour as compared with five-minute perfusion experiments points strongly against such a hypothesis. A suggestion which seems to be compatible with all the facts hitherto

Table 2. Analysis of Variance, Dose-response Lines, Difference between Slopes, and Potency Ratio with Fiducial Limits for Cetrimide, Phloridzin, and Cetrimide + Phloridzin in Half-hour Intestinal Perfusion Assays in the Mouse

	Cetrimide	Phloridzin	Cetrimide + phloridzin
Analysis of variance			
Source of variation:			
Linear regression	$P < 0.01$	$P < 0.001$	$P < 0.01$
Deviation from regression	$P > 0.05$	$P > 0.05$	$P > 0.2$
Dose-response line	$y = 62.55 - 20.17\,x$	$y = 51.46 - 22.51\,x$	$y = 16.11 - 8.79\,x$
x = log concentration			
Difference between slopes	2·34		13·72
	$P < 0.8$		$P < 0.01$
Potency ratio	4·8		10·45
Range of potency ratio at $P = 0.01$	26·7 – 1·9		70·2 – 3·8

established is that cetrimide may exert a conserving effect by displacing the phloridzin from 'silent receptors', where the drug has no action, to 'active receptors', so that the effective concentration is greatly increased. A further possibility is that the surface-active cetrimide may facilitate the penetration of phloridzin into the cell to its site of action. It may prove possible to put this hypothesis to the test by investigating other surfactants of different composition.

The present investigation raises a number of intriguing questions; for example, whether the synergistic action of cetrimide would also extend to the inhibitory effect of phloridzin on glucose re-absorption by the renal tubules, and whether phloridzin in turn would exhibit synergism with respect to the germicidal activity of cetrimide. Answers to such questions are being sought. In preliminary investigations there has been no obvious synergism between the two substances with regard to inhibition of intestinal alkaline phosphatase, though both seem to be equipotent in this respect.

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PARTIAL ACID-HYDROLYSIS OF A MUCOPOLYSACCHARIDE WITHOUT APPRECIABLE *N*-DEACETYLATION OF HEXOSAMINE

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IN the partial acid-hydrolysis of polysaccharides which contain residues of *N*-acetyl-hexosamine, scission of the glycosidic linkages is accompanied by *N*-deacetylation of the hexosamine residues[1-3]. This complication is avoided in partial acetolysis[4,5], but during subsequent *O*-deacetylation of the acetolysate, extensive degradation of the oligosaccharides may occur[5,6]. A partial acid-hydrolysate can be re-*N*-acetylated[7]; but we report here some results which show that this step may not always be necessary.

In a previous communication[8], one of us reported the conversion of inulin into oligosaccharides in high yield, by hydrolysis with water-soluble polystyrenesulphonic acid. This was accomplished by dialysing the oligosaccharides continuously from the reaction mixture as they were liberated; they thereby escaped further hydrolysis, since the acid was non-dialysable. It seemed likely that, if applied to a polysaccharide containing *N*-acetyl-hexosamine residues, the same technique would not only improve the yield of oligosaccharides, but also diminish the extent of *N*-deacetylation of the products.

To illustrate this possibility, methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was chosen as a model substance. A solution of this compound (20 mgm.) in 0·2 *N* hydrochloric acid (20 ml.) was heated at 86·5 ± 0·5°, and the liberation of 2-acetamido-2-deoxy-D-glucose was followed by a modification[9] of the Morgan–Elson method. The yield rose to a maximum of 0·77 mole/mole after 100 min., and then slowly decreased. The liberation of 2-amino-2-deoxy-D-glucose was also followed, and the yield of this product after 100 min. was 0·18 mole/mole. (This was measured as reducing power, using Nelson's reagents[10]; with the help of calibration curves, the concentrations of 2-amino-2-deoxy-D-glucose were determined, after subtracting from the total reducing power the contributions of the previously determined concentrations of 2-acetamido-2-deoxy-D-glucose.) Since methyl 2-amino-2-deoxy-β-D-glucopyranoside was hydrolysed to a negligible extent under the same conditions[6], it followed that the yield of 2-acetamido-2-deoxy-D-glucose after 100 min. would have been about 95 per cent, if it had been possible to remove this compound continuously from the reaction mixture as it was liberated.

Experiments were then carried out with a mucopolysaccharide prepared from hog gastric mucin. This material contained residues of fucose (10·2 per cent), galactose (23·5 per cent), 2-acetamido-2-deoxy-glucose (26·8 per cent) and 2-acetamido-2-deoxy-galactose (16·3 per cent); the remainder of the macromolecule consisted essentially of peptide material. (These analytical figures are subject to the usual errors involved in mucopolysaccharide analysis, which arise from incomplete release of monosaccharides by acid-hydrolysis[1-3].) The preparation of the water-soluble polystyrenesulphonic acid, and the general procedure for the hydrolysis, are described elsewhere[8].

To a solution of the mucopolysaccharide (300 mgm.) in water (40 ml.) was added polystyrenesulphonic acid (0·0404 *N*; 20 ml.), and 'Amberlite *IR*-120' (H^+ form) resin (5 gm.). (The 'Amberlite' resin was included to prevent gradual neutralization of the dilute acid by basic substances which were liberated during hydrolysis, and absorbed from the laboratory atmosphere. The 'Amberlite' resin was not an efficient catalyst when used alone, probably owing to the difficulty with which the large mucopolysaccharide molecules penetrated the resin particles.) The reaction mixture (*p*H 2·2) was dialysed at 86 ± 1° against distilled water (800 ml.). The initial effective area of membrane ('Visking' 32/32 tubing) was 240 cm.². At intervals, the dialysates were replaced by fresh distilled water[8]; the dialysates were evaporated separately, and the residues were weighed. The results are recorded in Table 1.

Table 1

Time (hr.)	1·5	4·0	7·0	11·0	21·0	50·0
Total weight dialysed (mgm.)	43·7	106·7	143·9	177·2	217·7	243·0

These residues were examined on paper chromatograms, which were developed in ethyl acetate/pyridine/water (10:4:3 v/v), and sprayed with silver nitrate–sodium hydroxide reagents[11]. The four above-named monosaccharides were detected, together with at least thirteen different oligosaccharides. Five of the oligosaccharides appeared from their chromatographic mobilities to be the five disaccharides previously isolated from human blood-group *A* substance in this laboratory[12]. Free hexos-

amine was not detected, either on these chromatograms or on similar chromatograms treated with ninhydrin[13]. None of the oligosaccharides produced a colour with ninhydrin. Most of the oligosaccharides gave a purple colour after treatment with alkali, followed by Ehrlich's reagent[14], indicating the presence of *N*-acetyl-hexosamine residues.

From the solution remaining in the dialysis bag, 76 mgm. of material was isolated, after neutralization and removal[8] of the polystyrenesulphonic acid and resin. This material consisted largely of free amino-acids, but also contained residues of hexosamine (4·2 per cent) and galactose (3·3 per cent). After taking into consideration the addition of the elements of water to each fragment during hydrolysis, and possible analytical errors, it was evident that about 90 per cent of the total carbohydrate moiety of the mucopolysaccharide had been converted into mono- and oligo-saccharides having their *N*-acetyl groups still intact.

In an attempt to rationalize this result, the rate of *N*-deacetylation of 2-acetamido-2-deoxy-D-glucose under the same hydrolytic conditions was studied. This compound (13·5 mgm.) was heated at 86 ± 1° in 0·0135 *N* polystyrenesulphonic acid (6 ml.). At intervals, samples were withdrawn and neutralized, and their content of 2-amino-2-deoxy-D-glucose was estimated with ninhydrin[15]. The results are shown in Table 2.

Table 2

Time (hr.)	1	2	3	5	7	10	13	16
N-deacetylation (per cent)	1·4	2·8	4·6	7·3	10·0	13·7	17·5	21·2

When the hyperbolic logarithms of the concentrations of 2-acetamido-2-deoxy-D-glucose were plotted against time, a straight line was obtained, having slope $k = 1{\cdot}5 \times 10^{-2}$ hr.$^{-1}$. Similarly, from the results in Table 1, the hyperbolic logarithms of the amounts of carbohydrate material remaining in the dialysis bag were plotted against time. An approximate straight line was obtained, having slope $K = 10{\cdot}5 \times 10^{-2}$ hr.$^{-1}$. Assuming that all the *N*-acetyl-hexosamine residues in the mucopolysaccharide were *N*-deacetylated at the same rate, irrespective of their state of combination, and that the proportion of these residues in the diffusible material remained constant throughout the hydrolysis, we can write $C_t = C_0 e^{-(k+K)t}$, where C_0 is the initial concentration of *N*-acetyl-hexosamine residues in the dialysis bag, and C_t is the concentration after time t. It may be deduced that the percentage *N*-deacetylation after time t is given by $\frac{100k}{k+K}(1-e^{-(k+K)t})$. When no provision is made for dialysis, $K = 0$, and the expression simplifies to $100(1-e^{-kt})$. In Table 3, these expressions have been used to calculate the percentage of *N*-deacetylation to be expected at various times, with and without the use of a membrane.

Table 3

Time (hr.)	*N*-deacetylation (per cent)	
	With dialysis	Without dialysis
2	2·7	2·9
5	5·7	7·2
10	8·8	13·8
20	11·4	26·0
50	12·5	52·8
100	12·6	77·7
∞	12·6	100·0

The maximum possible extent of *N*-deacetylation to be expected when the membrane was used is seen to be 12·6 per cent. Although these assumptions are unlikely to be strictly valid, this calculated result accounts satisfactorily for the observed high yield of *N*-acetylated mono- and oligo-saccharides.

The residues obtained from the various dialysates were combined and separated into mono- and oligosaccharide fractions by selective displacement from a charcoal–'Celite' column[16]. The yield of oligosaccharides represented about 40 per cent of the original weight of the mucopolysaccharide. This yield was more than three times as great as the maximum yield which we were able to obtain by hydrolysis with mineral acid. Separation and identification of the oligosaccharides are now in progress, details of which will be reported later.

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INTERMEDIATE POOL BETWEEN AMINO-ACIDS AND GLOBIN IN DUCK BLOOD *IN VITRO*

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IF duck red cells, previously incubated *in vitro* in their plasma with 2-^{14}C glycine, are washed and re-incubated in unlabelled plasma, the activity of hæmoglobin increases with time (re-incubation time) according to an exponential law[1]. These data are shown in Table 1. We find that inside red cells there is a pool of radioactive substances which cannot be removed completely by washing and which are used for hæmoglobin synthesis. The following equation fits the experimental points:

$$\alpha = 1 - A \exp(-\lambda t) \qquad (1)$$

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Table 1

Experiment	Re-incubation time (hr.)	Globin a_G	Globin $\overset{*}{a}_G$	Hæm a_H	Hæm $\overset{*}{a}_H$	$\frac{\overset{*}{a}_G + \overset{*}{a}_H}{2} = a$	$\frac{a_H}{a_G} = R$	$\bar{R} \pm S.E.$
1	0	185	0·66	3,400	0·58	0·62	18·4	
	1·5	252	0·90	4,890	0·83	0·86	19·4	
	3·0	277	0·99	5,880	1·00	1·00	21·2	20·1 ± 1·0
	6·0	277	0·99	5,815	0·99	0·99	21·0	
	18	285	1·02	5,900	1·01	1·01	20·7	
2	0	97	0·63	2,050	0·66	0·64	21·2	
	1·0	136	0·89	2,750	0·89	0·89	20·2	
	2·0	141	0·92	2,790	0·90	0·91	19·8	20·3 ± 0·6
	4·0	155	1·01	3,020	0·97	0·99	19·5	
	8·0	152	0·99	3,180	1·03	1·01	20·9	

$a_G(H)$, carbon-14 specific activity in counts/min./mgm. carbon. $\overset{*}{a}_G(H)$ is normalized to 280 (5,865) in experiment 1 and to 153 (3,100) in experiment 2; error < 5 per cent.

where α is specific activity in arbitrary units; A is 0·38 (arbitrary units); λ is 0·8 hr.$^{-1}$. We make the following assumptions: (*a*) the pathway of globin synthesis is: amino-acids → precursor X → globin; (*b*) the turnover time is infinite related to the re-incubation time and turnover time of precursor X; (*c*) the globin synthesis is in steady state in our experimental conditions; (*d*) radioactive glycine is completely removed by washing.

Then the globin specific activity must increase with time according to the following equation, from which we can deduce information on the globin precursor, that is, on its turnover time:

$$\alpha_G = \alpha_G^0 + \frac{\tau_x}{\tau_G}\alpha_x^0 - \frac{\tau_x}{\tau_G}\alpha_x^0 \exp(-t/\tau_x) \qquad (2)$$

α_G is globin specific activity at time t of re-incubation; α_G^0 is globin specific activity at zero time of re-incubation; α_x^0 is globin precursor specific activity at zero time; $\tau_G = 1/\lambda_G$ is globin turnover time; $\tau_x = 1/\lambda_x$ is globin precursor turnover time.

Equation (2) can easily be related to equation (1).

Dintzis, Borsook and Vinograd[2] found evidence that microsomal particles are the template code for globin in rabbit reticulocytes. In particular they found that a proportion of the microsomes turns over rapidly. Therefore they performed experiments to prove that the amino-acid composition of this transient material in the microsomes is the same as that of hæmoglobin. They used the tracer technique and deduced that the results were compatible with the assumption of the role of microsomes in hæmoglobin synthesis, but not conclusive. Indeed, one result (the first in Table 2) supports the hypothesis, another (the third in Table 2) is conflicting and yet another (the second in Table 2) is not informative. For details the reader is referred to the original paper[2].

Following the findings of these authors, we have performed experiments in order to prove a precursor–product relationship between microsomes and globin in duck blood, incubated *in vitro*. In particular, we investigated whether the turnover time of the microsomes agrees with that of globin precursor as deduced from equations reported previously, and whether a radioactive material balance from microsomes to globin is verified.

Table 2. Molar Ratios of Leucine to Various Amino-acids

	Microsomes		Hæmoglobin	
	Total compound	Label	Total compound	Label
Histidine	3·7	2·2	1·8	1·9
Phenylalanine	2·5	2·1	2·3	2·0
Arginine	1·0	3·2	5·7	7·5

Heparinized duck blood with added sodium benzyl penicillin and dihydrostreptomycin sulphate was incubated in 200-ml. vessels for 20–40 min. at 38·5° C. in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. Labelled glycine (specific activity 1 mc./m.mole) and 300 mgm. of glucose per 100 ml. of blood were also added. At the end of incubation, the red cells were separated by centrifugation and washed four times at 4–5° C. with cold saline containing 1 per cent unlabelled glycine and once with saline only. The red cells were resuspended in unlabelled plasma taken from the same duck (1 volume of red cells to 1 volume of plasma). Six samples (~ 20 ml. each) were prepared and re-incubated for 0, 1, 2, 3, 7 and 18 hr. After incubation, the red cells were separated and washed four times with cold saline at 4–5° C. and once with cold *tris* buffer, *p*H 8·2. The red cells were frozen in a liquid-air bath and thawed twice, then they were mixed with 2 volumes of cold *tris* buffer and centrifuged at low speed. The supernatant was centrifuged at 15,000*g* for 20 min. and at 100,000*g* for 3 hr. The sediments were resuspended in 50 volumes of buffer and respun twice. After ultracentrifugation, the supernatant solution of hæmoglobin was dialysed at 0° C. for a week against distilled water in a 1-litre vessel with a flux of 15 litre/day. The hæm and globin were prepared from hæmoglobin in the usual way[3]. We measured the carbon specific activities of all separated materials[4].

The results are shown in Tables 3 and 4. The specific activities of the fraction collected at 15,000*g* (mitochondria fraction) and 100,000*g* (microsomal fraction) decrease during the first hours of re-incubation and then attain a constant value. As the specific activities of microsomes and mitochondria decrease we can be sure that labelled glycine is removed from duck cells by washings.

The specific activities decrease according to a monotonic asymptotic curve. Subtracting this asymptotic value from specific activities and plotting their logarithms against time, we obtain a straight line. We suggest the following hypothesis: mitochondria and microsomes incorporate 2-^{14}C glycine in a transient part which turns over rapidly and in another part which turns over slowly. The turnover times of mitochondria and microsome transient part, deduced from the slopes of these straight lines, are different, but not very significantly.

It can be seen that the slope is affected by concentration of reticulocytes. The turnover time deduced

Table 3

Experiment	Re-incubation time (hr.)	Globin a_G	Globin $\overset{\bullet}{a_G}$	Hæm a_H	Hæm $\overset{\bullet}{a_H}$	$\frac{a_G + a_H}{2} = a$	$\frac{a_H}{a_G} = R$	Details
1	0	534	0·76	8,900	0·74	0·75	16·7	a is fitted by $1-0·3\ \exp(-0·4\ t)$
	1	602	0·86	9,100	0·76	0·81	15·1	
	2	602	0·86	10,320	0·86	0·86	17·1	
	3	656	0·94	10,200	0·85	0·90	15·5	
	7	734	1·05	11,600	0·97	1·01	15·8	$\bar{R} = 16·4 \pm 1·1$
	18	670	0·96	12,300	1·03	1·00	18·4	
2	0	148	0·71	1,740	0·63	0·67	11·8	a is fitted by $1-0·33\ \exp(-0·7\ t)$
	1	178	0·85	2,300	0·83	0·84	12·9	
	2	196	0·94	2,530	0·91	0·92	12·9	
	3	210	1·01	2,530	0·91	0·96	12·0	
	7	206	0·99	2,780	1·00	1·00	13·5	$\bar{R} = 12·6 \pm 0·6$
	18	210	1·01	—	—	1·01	—	

For $a_G(H)$ and $a_G(H)$ see footnote of Table 1. Error < 5 per cent.
In experiments 2 and 3 we used blood taken from a duck bled a week before.

$\overset{\bullet}{a_G}$ was normalized to 702 in experiment 1, to 208 in experiment 2. $\overset{\bullet}{a_H}$ was normalized to 11,950 and 2,780.

Table 4

Experiment	Re-incubation time (hr.)	a_M = carbon-14 specific activity of microsomes in counts/min./mgm. carbon	a_{mt} = carbon-14 specific activity of mitochondria in counts/min./mgm. carbon	Details
1	0	2,535		a_M is fitted by $a_M = 1,700 + 840\ \exp(-0·4\ t)$
	1	2,210		
	2	2,094		
	3	1,996		
	7	1,650		
	18	1,754		
2	0	730	1,000	a_M is fitted by $a_M = 470 + 260\ \exp(-0·6\ t)$
	1	590	600	
	2	548	396	a_{mt} is fitted by $a_{mt} = 290 + 750\ \exp(-0·9\ t)$
	3	568	344	
	7	440	284	
	18	506	298	

Experiments 1 and 2 are related to those experiments in Table 3.

from this slope is higher if the concentration is low. Also the increase of globin and hæm specific activities are affected by the concentration. We found, in fact, a more rapid increase when concentration of reticulocytes is higher than normal.

The following conclusions can be drawn: the turnover time of the transient part of microsomes agrees within our experimental errors with the turnover time which we deduce for globin precursor, according to the hypothesis reported above. But the radioactive material balance between microsomes and globin is not verified. If we suppose concentration of microsomes is about 100 mgm./100 ml. of red cells and hæmoglobin is 30 gm./100 ml. red cells, the increment of globin activity is higher (10–20 times) than that deducible from activity lost by the microsomes.

Therefore, only one condition, which must be verified for a precursor-product relationship, is observed.

Two hypotheses can be drawn: (*a*) the amino-acid chains of the transient part of microsomes is removed from microsomes by our technique of preparation; (*b*) the transient part of microsomes is not globin.

Scheiger *et al.*[5] found that in rabbit red cells there is a non-protein nitrogen pool the amount of which increases if hæmoglobin synthesis is stopped. Following this finding, we postulated a peptide pool as hæmoglobin precursor and experimented to prove this hypothesis.

Duck blood (about 40 ml.) was incubated as reported above with 100 μc. of 2-^{14}C glycine for 2 hr. Then red cells were separated from plasma and washed four times with cold saline added to unlabelled glycine (1·5 per cent) and once with cold potassium chloride isotonic solution. Then the cells were hæmolysed in a liquid-air bath and thawed; the hæmolysate was added to two volumes of isotonic potassium chloride; the ghosts and nuclei were discarded by centrifugation. The supernatant was diluted to 150 ml. with distilled water and ultrafiltered at 0° C. We used ultracellular-filter ('Allerfeinst') purchased from Membranfilter-Gesellschaft, Sartorius-Werke A.G. and Co., Gottingen. Only molecules with molecular weights lower than 6×10^4 A.M.U. can be filtered through these membrane-filters. Indeed our ultrafiltered solution was colourless. The ultrafiltrate was lyophilized and dissolved in isotonic potassium chloride.

At the same time we carried out a preliminary investigation on the ability of duck red cell hæmolysate, free from ghosts and nuclei, to incorporate 2-^{14}C glycine. The globin was found to be radioactive. The experimental conditions were as follows: 20 ml. of hæmolysate, from 25 ml. of blood, were added to 10 ml. of amino-acid solution (composition in mgm. per 100 ml. distilled water : arginine 1·54, aspartic acid 2·50, hystidine 2·16, *iso*leucine 1·00, leucine 2·50, lysine 4·10, phenylalanine 2·8, threonine 1·35, tryptophan 0·45, valine 2·40), 1 mgm. of ferric chloride, 5 ml. of phosphate buffer 0·15 *M* (*p*H = 7·4) and glucose (50 mgm.). All other incubation conditions were as reported. After 18 hr., the incubation was stopped and globin prepared in the usual way.

Finally, a conclusive experiment, incubating the red cell hæmolysate with the ultrafiltrate, was performed. Three samples were taken in the first run and four

Table 5

Exp.	Sample	a_d	Molarities of unlabelled amino-acids		
			Glycine	Serine	Alanine
1	1	162 ± 4	—	—	$0{\cdot}9 \times 10^{-3}$
	2	176 ± 4	$0{\cdot}25 \times 10^{-2}$	$0{\cdot}40 \times 10^{-2}$	$0{\cdot}9 \times 10^{-3}$
	3	182 ± 3	$0{\cdot}5 \times 10^{-1}$	$0{\cdot}80 \times 10^{-1}$	$0{\cdot}9 \times 10^{-3}$
2	1	72 ± 4	—	—	$0{\cdot}9 \times 10^{-3}$
	2	72 ± 5	$0{\cdot}5 \times 10^{-2}$	$0{\cdot}38 \times 10^{-2}$	$0{\cdot}9 \times 10^{-3}$
	3	64 ± 4	$0{\cdot}52 \times 10^{-1}$	$0{\cdot}38 \times 10^{-1}$	$0{\cdot}5 \times 10^{-1}$
	4	66 ± 2	$1{\cdot}06 \times 10^{-1}$	$0{\cdot}76 \times 10^{-1}$	$1{\cdot}0 \times 10^{-1}$

a = specific activity in dis./min./mgm.

in the second. They were different for amounts of inactive glycine, serine and alanine, carriers added. Except for 2-^{14}C glycine, which was not added, all other conditions were the same as described for hæmolysate incubation experiments.

From the data reported (Table 5) the following conclusions can be deduced: (*a*) Globin can absorb radioactivity from labelled ultrafiltered substances. (*b*) This radioactivity taken up is not due to free radioactive glycine or serine or alanine, which can be formed from glycine conversion[3], because the added carriers were not effective in reducing globin specific activity. Therefore, we suggest a free peptide pool as globin precursor in duck blood *in vitro*.

Further experiments are in progress to prove precursor product relationships between the transient part of the microsomes and these peptides and globin, and to find the molecular weights of these peptides. Indeed, we know only an upper limit, because almost all radioactivity of ultrafiltrate can dialyse.

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DIDEOXYSUGARS OF *PASTEURELLA PSEUDOTUBERCULOSIS*-SPECIFIC POLYSACCHARIDES, AND THE OCCURRENCE OF ASCARYLOSE

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THE isolation and composition of the type-specific polysaccharides of the five serotypes of *Pasteurella pseudotuberculosis* have previously been described[1]. The identity of the 3,6-dideoxy-hexoses present in these materials, and to which the serological specificity may be largely due, was, however, left in some doubt.

Sugars of the 3,6-dideoxy-hexose series (paratose, abequose, colitose and tyvelose) are known to occur in polysaccharides of Enterobacteria[2], where they occupy the positions of non-reducing end-group residues and are the specific serological determinants responsible for some 'antigens' of the Kauffmann–White scheme. One other sugar of this series, ascarylose, is known to occur in the egg cell membranes of *Ascaris* worms[3].

It was previously reported that *P. pseudotuberculosis* type II polysaccharide contains abequose (3,6-dideoxy-D-*xylo*-hexose); this was based on chromatographic data only. This identification has been confirmed in the following way, using a serological cross-reaction which has long been known[4]. Specific polysaccharide extracted from *Salmonella salinatis* (which contains abequose, responsible[5] for Kauffmann–White 'antigen' 4) was adsorbed on to sheep erythrocytes[6], which thus became agglutinable not only by homologous *Salmonella Gp. B* antisera but also by *P. pseudotuberculosis* type II antiserum. Similarly, erythrocytes sensitized with *P. pseudotuberculosis* type II polysaccharide could be agglutinated by both antisera. The hæmagglutination of both kinds of sensitized sells by the heterologous antisera could be inhibited by abequose (isolated fron *S. salinatis* polysaccharide) but not by any other sugar tested, including those most closely related structurally to abequose (for example, D-fucose, D-galactose, D-glycero-D-galactoheptose, tyvelose). Sufficient dideoxy-sugar was isolated from *P. pseudotuberculosis* type II polysaccharide to test in this system, and inhibition was again obtained, leaving no doubt that this sugar is abequose.

It was previously reported[1] that *P. pseudotuberculosis* type IV polysaccharide contains tyvelose (3,6-dideoxy-D-*arabino*-hexose), but again only on chromatographic evidence. It is already known[7] that this type is related serologically to *Salmonellae* of group *D*, due to Kauffmann–White antigen 9, and that this 'antigen' is due to tyvelose end-groups in the group *D* specific polysaccharide[8]. The cross-reaction could be demonstrated readily by hæmagglutination as already described, but in this case the reactions could not be inhibited by tyvelose (isolated from *S. typhosa* polysaccharide), ascarylose (its optical enantiomorph), or any other sugar tested. The dideoxy-sugar was therefore isolated from *P. pseudotuberculosis* type IV polysaccharide; material was hydrolysed for 20 min. in *N* sulphuric acid at 100°, neutralized with barium hydroxide and the salt-free hydrolysate concentrated to a thin syrup. The leading component was isolated by elution from chromatograms run on Whatman 3-mm. paper irrigated with butanol/pyridine/water (6 : 4 : 3); the eluates were concentrated to a syrup, which gave a strong positive rotation. Since ascarylose has a negative rotation, the *P. pseudotuberculosis* type IV sugar is tyvelose.

The polysaccharide of *P. pseudotuberculosis* type V contains a sugar which cannot be distinguished

chromatographically from tyvelose[1]. In view of the different specificities of the type IV and V materials, the sugar would be either tyvelose differently linked in the two instances or alternatively the type V material might contain the optical isomer ascarylose (3,6-dideoxy-L-*arabino*-hexose). The dideoxy-sugar of type V polysaccharide was isolated chromatographically as previously described and the syrup, after filtration through charcoal, gave a strong negative rotation ($[\alpha]_D = -20°$, about 2 in water), approaching that of crystalline ascarylose ($[\alpha]_D = -25°$). This identity was confirmed by preparing the dideoxy-hexitol[2]; the syrup (approx. 25 mgm.) was reduced in aqueous solution with $NaBH_4$ (25 mgm.) and left at room temperature overnight, excess $NaBH_4$ was then destroyed with a little *N* acetic acid. The solution was evaporated to dryness over phosphorus pentoxide and sodium hydroxide pellets and the residue extracted with methanol; the methanol solution was dried and extracted several times with boiling acetone, the acetone solution reduced in volume and left in the cold to crystallize. After recrystallization from acetone the dideoxy-hexitol gave $[\alpha]_D = +38 \pm 3°$ (in methanol) and had an m.p. of 112–113°. This is clearly the optical isomer of tyvelitol and the *P. pseudotuberculosis* type V sugar is ascarylose.

For polysaccharides of *P. pseudotuberculosis* types I and III, chromatograms indicated that the dideoxy-sugars differed from abequose and tyvelose (or their optical isomers colitose and ascarylose), and migrated in the position of paratose (3,6-dideoxy-D-*ribo*-hexose)[3]. The chromatographic separation of these sugars differed from abequose and tyvelose (or their separation could be obtained on paper electrophoresis in borate buffer[10]. In this system the sugars could be separated completely from one another in a 2-hr. run, with $R_{rhamnose}$ values of 1·03 for abequose/colitose, 1·22 for tyvelose/ascarylose and 1·38 for paratose. The types I and III dideoxy-sugars gave $R_{rhamnose}$ 1·38 but the different specificities of the polysaccharides required a demonstration of whether the sugars were indeed identical or again a pair of optical isomers.

The sugars were isolated as described above. The specific rotation of paratose is only +10° and the sugar syrups both gave positive rotations; with the amount of material available, however, the absolute readings were too small to be convincing. From the solutions, dideoxy-hexitols were prepared by reduction with $NaBH_4$ as described above, and in both cases the hexitols gave $[\alpha]_D$ approx. −20° (paratitol $[\alpha]_D$ −18°). It is clear, therefore, that paratose is present in both type I and III polysaccharides and is presumably linked in a different way in each instance to give the different specificities.

The situation is thus, that all the known 3,6-dideoxy-hexoses occur in bacterial polysaccharides[11] and these include all the configurations except the L-*ribo*- and D- and L-*lyxo*- forms.

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SIALIC ACIDS AND THE ELECTROKINETIC CHARGE OF THE HUMAN ERYTHROCYTE

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It has long been recognized that strongly acidic groups are responsible for the net negative charge of the erythrocyte. Furchgott and Ponder[1] suggested that the charge was derived from a phospholipid, possibly cephalin. Winkler and Bungenberg de Jong[2] came to similar conclusions from charge reversal spectra work.

Recent evidence indicates that the electrokinetic behaviour of the erythrocyte may derive from mucosubstances. Hirst[3] suggested, from erythrocyte virus adsorption and elution studies, that the sites of adsorption may have a mucoprotein character. Hanig[4] showed that adsorption of virus with concomitant agglutination of the red cells followed by elution of the virus was associated with a very marked decrease in the microelectrophoretic mobility of the red cells. Stone and Ada[5] examined more fully the relationship between changes in the microelectrophoretic mobility of red cells and their agglutination after treatment with viral enzymes and discussed the changes in terms of the receptor gradient of Burnet[6].

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The specificity of an enzyme derived from *Vibrio cholerae* cultures (receptor-destroying enzyme) was examined by Gottschalk and Lind[7], and afterwards recognized as a glycosidase by Gottschalk[8] and termed 'neuraminidase' by him[9] because of its ability to liberate acylated neuraminic acids by cleavage of an α-glycosidic linkage[10].

Klenk and Uhlenbruck[11] have found that the receptor-destroying enzyme will liberate acylated neuraminic acids from erythrocyte stroma of various mammalian species. Furthermore, Klenk and Lempfrid[12] found that the sialic acid liberated by the receptor-destroying enzyme from human erythrocyte stroma was N-acetylneuraminic acid (NANA). Klenk[13] suggested that the negative charge of the erythrocyte could be ascribed to an acylated neuraminic acid.

Trypsin releases a sialomucopeptide[14] which is thought to be related to the decrease in the electrophoretic mobility of the treated erythrocyte[15,16].

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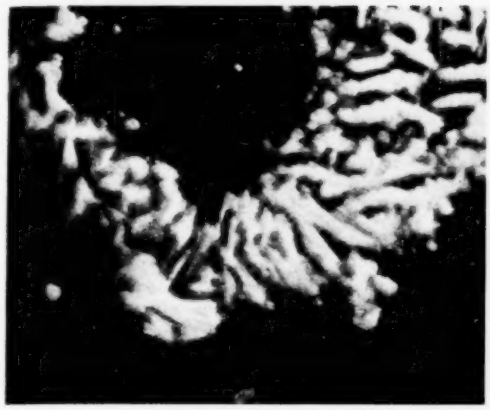

Fig. 1. Photograph of sialic acid crystallized from the supernatant of erythrocytes treated with receptor-destroying enzyme using methanol/diethyl ether/light petroleum mixture. Dark ground illumination. (× *c.* 150)

It is shown here that the decrease in erythrocyte charge observed after treatment by crystalline neuraminidase is brought about by the release of an N or O,N-acetylated neuraminic acid, the carboxyl group of which is in consequence removed from the electrophoretic plane of shear. The reduction in the electrokinetic charge of the erythrocyte is shown to be essentially a loss of anionic groups rather than the generation of a significant proportion of cationic groups.

Standard washed human erythrocytes[16] phenotypes (*ARh*+ve, *ORh*+ve and −ve) were used in the experimental work. Packed red cells (about 1 c.c. per experiment) were treated with receptor-destroying enzyme prepared according to the method of Ada and French[17]. The receptor-destroying enzyme solution 1 vol. (activity 1,500 units per ml. [1 mgm. receptor-destroying enzyme protein = 10^7 units] in 0·145 *M* aqueous sodium chloride : 0·005 *M* aqueous calcium chloride buffered to *p*H 7 with 0·5 *M* aqueous sodium bicarbonate) was incubated for 1 hr. at 37° with 1 vol. of human erythrocytes (packed 2,000*g* for 15 min.). The erythrocyte suspensions treated with receptor-destroying enzyme were centrifuged (2,000*g* for 10 min.) and the supernatant fluids removed for analysis.

The receptor-destroying enzyme-treated and the appropriate control red cells were given a standard wash in 0·145 *M* aqueous sodium chloride[18] and the treated red cells examined by microelectrophoresis[18]. Samples of standard washed red cells subjected to the receptor-destroying enzyme were treated with a 2 per cent v/v acetaldehyde solution in 0·145 *M* aqueous sodium chloride buffered to *p*H 7·5 with 0·5 *M* aqueous sodium bicarbonate solution[19]. The receptor-destroying enzyme/acetaldehyde-treated red cells were stored for not less than twenty days, given a standard wash in 0·145 *M* aqueous sodium chloride solution and then examined by microelectrophoresis. The *p*H of the 0·145 *M* aqueous sodium chloride solution was adjusted by means of isotonic aqueous hydrochloric acid or sodium hydroxide.

The supernatant fluid from the red cells treated with receptor-destroying enzyme was deproteinized by the addition of 1 vol. of 10 per cent w/v aqueous trichloroacetic acid solution to 1 vol. of supernatant fluid. After flocculation and centrifugation (2,000*g* for 10 min., 4° C.) the clear supernatant fluid was assayed for sialic acids by the resorcinol-hydrochloric acid method of Svennerholm[20] using NANA as a standard. Sialic acids found in the control supernatant fluids were negligible.

The sialic acid released was characterized microscopically by its crystalline form and by paper chromatography. The supernatant fluid from 2-c.c. packed erythrocytes treated with receptor-destroying enzyme was deproteinized with trichloroacetic acid, filtered through a Whatman No. 50 paper and the clear filtrate extracted with diethyl ether. The aqueous phase was not desalted so as to avoid unnecessary loss of any labile sialic acids, but was concentrated directly under reduced pressure at 50° C. and an equal volume of methanol added. The sialic acid was crystallized by adding a few drops of diethyl ether : light petroleum (60–80° C.) (1 : 2 by vol.) mixture and leaving at 4° C. for 24 hr. Clusters of fluffy needle-like crystals (about 200 μgm.) were obtained (Fig. 1). The mixture from which sialic acids could be crystallized was examined by descending paper chromatography (Whatman No. 1 paper) using: (*a*) butan-1-ol : acetic acid : water (4 : 1 : 5), organic phase; (*b*) butan-2-ol : acetic acid : water (4 : 1 : 5). The air-dried chromatograms were dipped in orcinol–trichloroacetic reagent (3 : 5-dihydroxytoluene (0·5 gm.) trichloroacetic acid (15 gm.) dissolved in water-saturated butan-1-ol (100 ml.)) and developed at 105° C. The chromatographic behaviour of NANA (in solvent *b*) was examined in the presence of the same quantity of sodium chloride as in the experimental samples (Fig. 2).

It has been suggested[21] that the decrease in the electrophoretic mobility of erythrocytes after treat-

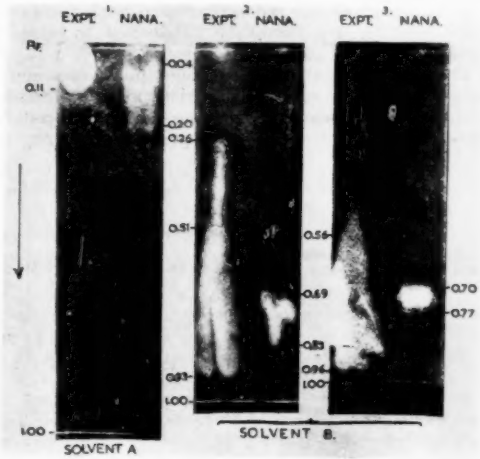

Fig. 2. Photostat copies of chromatograms developed with orcinol/trichloroacetic acid reagent

(1) Solvent *A* (butan-1-ol: acetic acid: water (4 : 1 : 5) descending); material released from erythrocytes by the receptor-destroying enzyme (R_F 0·11); N-acetylneuraminic acid (50 μgm.) (R_F 0·04–0·20)

(2) Solvent *B* (butan-2-ol: acetic acid: water (4 : 1 : 5) descending); material released from erythrocytes by receptor-destroying enzyme (R_F 0·26–0·51) (0·51–0·93); N-acetylneuraminic acid (50 μgm.) (R_F 0·69–0·83)

(3) Solvent *B*. N-acetylneuraminic acid (50 μgm.) and sodium chloride (12 mgm. equiv. to sodium chloride present in Exp. 2 above). (R_F 0·56–0·96); N-acetylneuraminic acid (50 μgm.) (R_F 0·70–0·77)

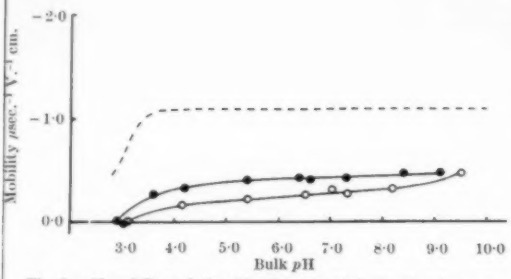

Fig. 3. *p*H-mobility relationships for untreated, receptor-destroying enzyme-treated, and receptor-destroying enzyme/acetaldehyde-treated red cells in 0·145 *M* aqueous sodium chloride at 25° C. – – –, untreated cells; ○, red cells treated with receptor-destroying enzyme; ●, red cells treated with receptor-destroying enzyme/acetaldehyde

ment with the *PR8* strain of influenza virus, results from the appearance of cationic groups. The titration studies of Curtain[22] on urinary inhibitor, which showed that treatment of this inhibitor with receptor-destroying enzyme yielded a substance possessing cationic groups of about *p*K 11, supported this view. Recent work on the specificity of neuraminidases by Gottschalk[5] has shown that the receptor-destroying enzyme is an α-glycosidase, and therefore that its action is unlikely to yield cationic groups.

Aldehyde treatment of normal human erythrocytes produces no detectable change in their electrophoretic behaviour[19], which from the specificity of aldehydes for potential cationic groups[22] suggests the absence of cationic groupings effective at the electrophoretic slip plane[19]. The relatively small change in mobility of human erythrocytes subjected to receptor-destroying enzyme after treatment with acetaldehyde, in conjunction with constancy of the isopotential *p*H (Fig. 3) indicates that only a small part of the reduction in charge of the red cell treated with receptor-destroying enzyme could be attributed to cationic groups.

The possibility that the changes in electrokinetic behaviour of the red cell observed after treatment with receptor-destroying enzyme could be brought about by the irreversible adsorption of the enzyme or by impurities in the receptor-destroying enzyme is considered to be unlikely for the following reasons: (1) Purification of the crystalline receptor-destroying enzyme normally involves its adsorption and facile elution from packed washed human erythrocytes. (2) The receptor-destroying enzyme has been shown from hæmagglutination studies[24] to be eluted from red cells and the red cells to be incapable of further adsorption of enzyme[24]. (3) There is not likely to be sufficient material present as an impurity in the receptor-destroying enzyme (total protein 0·1 μgm./ml.) to produce significant coverage of 10^{10} red cells.

The *p*H-mobility relationships in 0·145 *M* aqueous sodium chloride for normal, receptor-destroying enzyme treated and receptor-destroying enzyme/acetaldehyde treated human red cells given in Fig. 3 show: (1) For the normal red cell the *pKa* of the surface ionogenic groups is at about 2·8 (the *pKa* of NANA is 2·6 (ref. 25)). (2) For the receptor-destroying enzyme/acetaldehyde-treated red cells there is only a small increase in the mobility compared with the corresponding red cells treated with receptor-destroying enzyme. The cationic character of the treated red cell is too small to account for the reduction in mobility of the normal red cell on treatment with receptor-destroying enzyme; these observations are consistent with those of Stone and Ada[5].

Assuming the sialic acid released by the action of the receptor-destroying enzyme to be free NANA[12] of mol. wt. 309, and when bound in the membrane of the erythrocyte that its carboxyl group is effective at the electrophoretic plane of shear, the theoretical quantity of a sialic acid expected to be released per 10^{10} human erythrocytes for a given reduction in surface charge density was calculated. The carboxyl group of each sialic acid moiety when bound in the red-cell membrane was assumed to correspond to one electron charge on the surface of the erythrocyte. The red-cell charge densities were calculated from the Gouy–Chapman equation which at 25° C. for uni-univalent electrolytes reduces to:

$$\sigma = 3{\cdot}52 \times 10^{4}.I^{1/2}.\sin h(\zeta/51{\cdot}3)$$

where σ is the charge density in e.s.u. cm.$^{-2}$, I is the ionic strength[26] and ζ the zeta potential in mV. The

Table 1

Subject	Phenotype	Experiment	Total No. of erythrocytes per experiment	Erythrocyte: Mobility μsec.$^{-1}$ V.$^{-1}$ cm.	Erythrocyte: Charge density σ e.s.u. cm.$^{-2}$	Reduction in charge density Δσ e.s.u. cm.$^{-2}$	No. of electrons lost per 10^{10} erythrocytes	Theoretical yield of NANA per 10^{10} erythrocytes (μgm.)	Experimental yield of NANA per 10^{10} erythrocytes (μgm.)	Ratio experimental/theoretical yield of NANA	Remarks
1	*ARh* + ve	R.D.E.	1×10^{10}	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	89·2	2·13	Red cell counts and hæmatocrits performed in duplicate on each system using standard techniques
		Control	1×10^{10}	−1·08	3,680	—	—	—	—	—	
2	*ORh* + ve	R.D.E.	$1{\cdot}2 \times 10^{10}$	−0·39	1,311	2,369	$8{\cdot}03 \times 10^{16}$	41·2	81·9	1·98	
		Control	$1{\cdot}2 \times 10^{10}$	−1·08	3,680	—	—	—	—	—	
3	*ORh* + ve	R.D.E.	1×10^{10}	−0·82	2,753	927	$3{\cdot}14 \times 10^{16}$	16·1	42·8	2·66	
		Control	1×10^{10}	−1·08	3,680	—	—	—	—	—	
4*a*	*ORh* − ve	R.D.E.	$8{\cdot}4 \times 10^{9}$	−0·32	1,074	2,606	$8{\cdot}83 \times 10^{16}$	45·3	92·8	2·05	See below
		Control	$8{\cdot}4 \times 10^{9}$	−1·08	3,680	—	—	—	—	—	
4*b*	*ORh* − ve	R.D.E. 1	$1{\cdot}2 \times 10^{10}$	−0·41	1,377	2,303	$7{\cdot}81 \times 10^{16}$	40·0	73·2	1·83	Repeat experiment on same subject, 18 days interval between 4*a* and 4*b*
		2	$1{\cdot}2 \times 10^{10}$	−0·48	1,611	2,069	$7{\cdot}01 \times 10^{16}$	36·0	68·0	1·89	
		Control	$1{\cdot}2 \times 10^{10}$	−1·08	3,680	—	—	—	—	—	
5	*ORh* + ve	R.D.E. 1	$6{\cdot}85 \times 10^{9}$	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	108·1	2·59	
		2	$6{\cdot}85 \times 10^{9}$	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	96·2	2·30	
		Control	$6{\cdot}85 \times 10^{9}$	−1·08	3,680	—	—	—	—	—	

R.D.E., receptor-destroying enzyme.

2·18
Mean ratio exp./theor. yield of NANA

Gouy–Chapman equation may yield low charge densities for an ion-penetrable system, such as a red cell. Haydon[27] has pointed out that for such a system the following equation would be more likely to yield a reliable value for σ:

$$\sigma = (1 + \sqrt{1 - \alpha})\, 3{\cdot}52 \times 10^4 . I^{1/2} . \sinh(\zeta/51{\cdot}3)$$

where α is the fraction of the total space within the surface which is not available for counter ions. For $\alpha = 1$ the Gouy–Chapman equation is obeyed; for $\alpha = 0$ the actual surface charge density will be twice that found from the Gouy–Chapman equation. At present, in the case of a red cell, only an arbitrary value can be ascribed to α.

The experimental yields of sialic acids are given in Table 1 and are about twice those expected from the Gouy-Chapman theory. Even though the overall accuracy of the experimental results is unlikely to be better than about ± 20 per cent, because of the error involved in red cell counts and the unavoidable slight hæmolysis during the treatment with receptor-destroying enzyme, not all the variation between the experimental results is likely to be accounted for by this error. The Haydon factor α is unlikely to account completely for the discrepancy between the theoretically calculated yields of sialic acid and those found experimentally. The divergence between the theoretical and experimental figures indicates that the sites of neuraminidase action may not be sterically localized in the regions of the cell surface where the carboxyl group of the sialic acids are electrokinetically fully effective. It is therefore probable that some sialic acid has arisen from regions deep to the electrophoretic plane of shear and that this portion of the total sialic acid liberated by the receptor-destroying enzyme may depend on stereospecific factors and therefore be liable to variation from person to person, thus possibly accounting for the variation in the experimental results.

The chromatographic behaviour of the material released from the red cell by treatment with receptor-destroying enzyme (Fig. 2) is indicative of NANA with possibly some O,N-acetylated neuraminic acids present; the NANA could have originated from the decomposition of a labile O,N-acetylated neuraminic acid.

The results described in this article indicate that the dominant ionogenic group at the surface of the human erythrocyte is the carboxyl group of a bound N or O,N-acetylated neuraminic acid.

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PARTITION OF URINE NITROGEN IN KWASHIORKOR

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IT has been known for many years that the level of dietary protein intake greatly affects the partition of nitrogen in the urine[1]. In particular, low protein diets are associated with reduced relative excretion of urea nitrogen and with increased relative amounts of ammonia and of "undetermined nitrogen"[1,2]. Recent investigations[3] have shown that there is an increase in the excretion of free amino-acids in severe protein deficiency in children (kwashiorkor), from which it may be inferred that part of the increase in the "undetermined nitrogen" of urine in subjects on poor protein diet is caused by increased amounts of free amino-acid nitrogen. During investigation of the excretion of free amino-acids in kwashiorkor, we observed that in many of our patients the formol titration method of determining amino-acids in urine showed a much higher degree of amino-aciduria than is evident from chromatograms of the free amino-acids of the urines. It was suspected that there may be an increased excretion of other urine constituents which are estimated as 'amino-acids' by the formol titration procedure. We therefore examined the amino-acids of hydrolysed urines and we present here a preliminary report on observations on the partition of urine nitrogen and the excretion of bound amino-acids in kwashiorkor.

Urine ammonia was estimated by direct nesslerization of diluted urine: the results do not differ significantly from values obtained by the microdiffusion method of Conway[4]. Urea plus ammonia was determined by nesslerization after incubating with urease. Urea was obtained by difference. Total nitrogen was determined by the micro-Kjeldahl method. Creatinine, and creatinine plus creatine, were

measured by the alkaline picrate method, before and after boiling with picric acid[5]. Creatine was obtained by difference. Uric acid was determined by the uricase method[6] and amino-acid nitrogen by formol titration with subtraction of ammonia nitrogen[7]. Two-dimensional chromatography was carried out in butanol/pyridine/water and phenol/ammonia/water according to the procedure described by Smith[8]. For free amino-acids, electrolytically desalted urine was applied to the paper, aliquots containing 250 μgm. nitrogen being used for normal urines. Free plus bound amino-acids were examined after the urine was hydrolysed by refluxing 1 ml. of protein-free urine with 9 ml. of 6 *N* hydrochloric acid for 24 hr., removing the hydrochloric acid by evaporating to dryness and re-dissolving the residue in 4 ml. water and desalting. A four-fold dilution of the original urine was thus made during this process. The excretion of bound amino-acids was assessed by noting qualitative and quantitative changes in the chromatogram of the hydrolysed urine as compared with that of unhydrolysed urine containing an equivalent amount of total nitrogen.

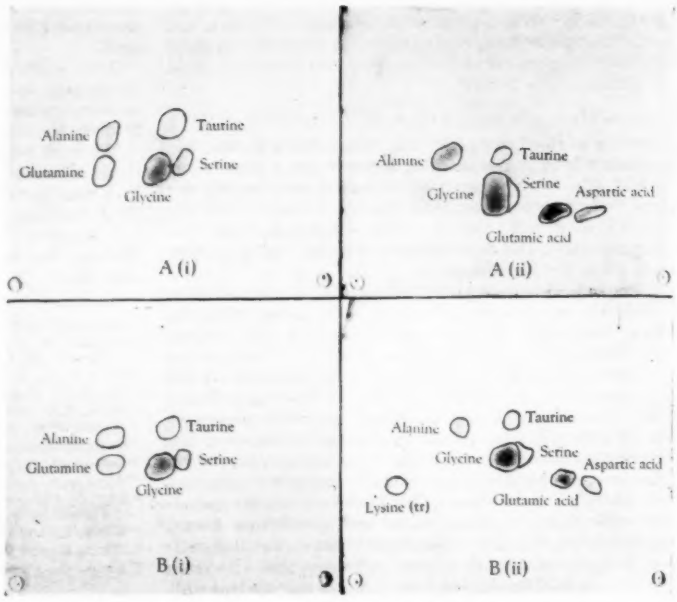

Fig. 1. Amino-acid excretion in healthy children
A (i) and *B* (i), free amino-acids in urine.
A (ii) and *B* (ii), free plus bound amino-acids after acid hydrolysis. All samples contain 250 μgm. nitrogen.

The partition of urine nitrogen in healthy Nigerian children presumed to be on normal protein diets, in healthy Nigerian children presumed to eat low protein diets and in children with kwashiorkor are shown in Table 1. The results confirm earlier observations that in kwashiorkor there is an increase in the relative amounts of ammonia and of amino-acids. They also show that there is an increase in the relative excretion of creatine and of uric acid. It should be emphasized, however, that the increases are in relative amounts, and do not necessarily indicate increased daily output. In kwashiorkor, urine volume is suppressed to about half to one-third of normal, and since the concentration of total nitrogen is also reduced to about a half, only those substances which show an increase in relative amounts of more than approximately four times are likely to show increase in absolute amount per 24 hr.

The relative excretion of urea is very significantly less than normal: there is very little change in the relative concentration of creatinine. The absolute amounts of these two substances per 24 hr. are very much below normal.

Table 1. Partition of Nitrogen in Urine in Healthy Controls and in Kwashiorkor
Values expressed as percentage of total nitrogen, unless stated otherwise

	Controls: *A* (12)	Controls: *B* (13)	Kwashiorkor (25)
Total nitrogen (gm./litre)	8·0	7·0	3·8
Ammonia	4·5	7·5	20·2
Urea	79·5	54·9	41·2
Creatinine	4·1	3·4	3·8
Creatine	0·2	—	2·5
'Uric acid'	3·5	—	6·8
'Amino-acids'	3·2	—	25·3

Controls *A*: Healthy children, being children of African members of the academic staff of the University College and presumed to eat normal protein diets.
Controls *B*: Healthy children from Imesi, a village about 100 miles from Ibadan and presumed to eat the traditional low-protein diet.

Fig. 1 shows photographs of chromatograms of free and total amino-acids in two healthy children on normal protein diets. Serine, glycine, taurine, alanine and histidine (sometimes methyl histidine) are usually present, but essential amino-acids (leucine plus *iso*leucine plus phenylalanine in one spot, valine plus methionine plus tryptophan in one spot, arginine, lysine, tyrosine, tryptophan) and other spots (asparagine, ornithine) are occasionally observed, though when present they are excreted in only trace amounts. The hydrolysed urines usually show an increase in the intensity of glycine and alanine and the appearance of aspartic acid and glutamic acid. Glutamine is destroyed in the process. Traces of other amino-acids may appear in some cases.

Kwashiorkor urines usually show varying degrees of amino-aciduria (Fig. 2). Chromatograms of free amino-acids comparable with those for normal children can usually be produced by spotting a volume of urine containing 2–15 (usually 4) times less nitrogen than we apply in the case of normal urines, indicating that there is an approximately four-fold increase in the proportion of free amino-acid nitrogen to total nitrogen in the urine of kwashiorkor patients. Essential amino-acids are not involved in this increase: in the majority of cases essential amino-acids (with the exception of lysine) could not be identified in chromatograms of the urine. The almost invariable over-excretion of β-amino*iso*butyric acid and the appearance of ethanolamine in several cases are other features of the amino-aciduria of kwashiorkor. Bound amino-acids also show considerable increase. The increase in bound amino-acids in most cases appears to be more than the increase in the excretion of free amino-acids. Clinical recovery is associated with

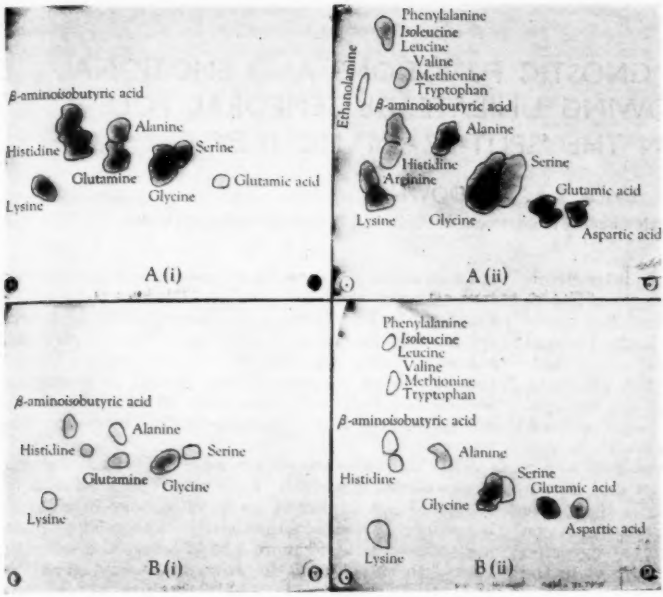

Fig. 2. Amino-acid excretion in a child with kwashiorkor
A (i), free amino-acids, and *A* (ii), free plus bound amino-acids, after acid hydrolysis. Urine spotted contained 250 μgm. nitrogen.
B (i), free amino-acids, and *B* (ii), free plus bound amino-acids, after acid hydrolysis. Urine spotted contained 62·5 μgm. nitrogen.
When this is compared with Fig. 1, it shows that there was more than a four-fold increase in both free and bound amino-acids.

return of the excretion of free and total amino-acids to normal, but over-excretion of β-amino*iso*butyric acid may persist for some time.

Creatine, creatinine and uric acid were also determined in blood serum. The results are shown in Table 2.

Table 2. SERUM CONCENTRATION IN MGM./100 ML.

Determination	Control children (30)	Kwashiorkor (25)
Creatine	1·41 ± 0·38 (0·8 – 2·3)	1·50 ± 0·79 (0·4 – 3·6)
Creatinine	0·75 ± 0·18 (0·5 – 1·2)	0·60 ± 0·23 (0·4 – 1·0)
'Uric acid'	3·95 ± 1·3 (1·6 – 6·3) In only 1/30 serum-level > 6·0 mgm./100 ml.	8·5 ± 4·3 (1·3 – 23·3) In 17/25 serum-level > 6·0 mgm./100 ml.

The increased output of ammonia is probably the result of increased excretion of organic acids[9] and of failure of the kidneys to produce acid urine[10]. Dean[11] has suggested that the excretion of large amounts of ammonia may show that ammonia is being produced that cannot be carried to urea because the xanthine–citrulline–arginine cycle is defective: it should be pointed out, however, that normally urinary ammonia is not derived from blood ammonia, but is produced in the kidneys as a defensive mechanism for body cation. The creatinuria is associated with increased breakdown of muscle. The fall in total creatinine excretion is probably due to the diminished mass of muscle; decreased renal clearance of creatinine has been postulated as an additional factor, but, as our results show that there is no increase in creatinine-level in plasma, we do not consider this likely. The etiology of the amino-aciduria of kwashiorkor has been discussed in a previous communication[3], in which it was also shown that ethanolaminuria is caused by deficiency of methyl donors.

The daily urinary excretion of uric acid is not a very sensitive index of the total uric acid turnover, because extra-renal excretion eliminates a considerable portion of the uric acid produced each day[12]. The level of uric acid in the plasma is a more reliable index, and the substantial rise in serum uric acid observed in kwashiorkor points clearly, in the absence of severe renal damage, to over-production of uric acid in this disease. Dean[11] has observed that in kwashiorkor the urine contains increased quantities of purine metabolites other than uric acid; these purines contribute to the urine as much nitrogen as uric acid, and thus account for part of the "undetermined nitrogen". We have been able to account for practically all the "undetermined nitrogen" without allowing for these purines; it may well be that uricase is not specific for uric acid and that part of the increase which we have noted is due to these abnormal purines. The accumulation of purine metabolites points, like the over-production of β-amino-*iso*butyric acid, to rapid cellular breakdown.

The bound amino-acids present in urine include peptides and amino-acids conjugated with other urine constituents. It can readily be demonstrated by treating, with biuret reagent, urine which has been de-colorized with activated charcoal and deproteinized with zinc sulphate and sodium hydroxide, that much of the increase in the bound amino-acids of kwashiorkor urine is due to increased peptide excretion. It is not yet clear whether this peptiduria is due to rapid tissue breakdown, or whether it is associated with failure of protein synthesis caused either by enzyme deficiencies or by deficiency of key amino-acids required to link the peptide chains together. Some of these peptides may be biologically active and may relate to the retention of fluid and to the circulatory collapse which sometimes supervenes in the course of severe kwashiorkor.

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CHANGES IN VISUAL GNOSTIC FUNCTIONS AND EMOTIONAL BEHAVIOUR FOLLOWING UNILATERAL TEMPORAL POLE DAMAGE IN THE 'SPLIT-BRAIN' MONKEY

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DURING the course of investigating inter-hemispheric transfer of visual discrimination habits in monkey (*Macaca mulatta*), it was found in three animals that transfer of monocularly learned visual discrimination habits did not occur following mid-sagittal division of optic chiasma, corpus callosum, anterior commissure and that part of the hippocampal commissure lying subjacent to the corpus callosum[1,2]. Transfer of these habits did occur in a fourth animal in which the anterior commissure remained intact although optic chiasma, corpus callosum and hippocampal commissure had been sectioned. Since the anterior commissure is the major commissure between the temporal poles[3-5,7] these observations suggested that the temporal poles might play some part in the transfer of visual gnostic behaviour.

A right unilateral temporal pole resection was carried out in an animal (*D*-26) that had previously completed a visual discrimination training sequence following mid-sagittal sectioning of optic chiasma and cerebral commissures. Following a recovery period of two weeks duration, the eye ipsilateral to the temporal pole lesion was left open and the opposite eyelids sutured shut. There appeared immediately many of the behavioural patterns described by Kluver and Bucy[6,7] in animals with bilateral temporal pole removals. The animal seemed unable to recognize edible from non-edible objects by visual cues alone; it would pick up the objects and spend considerable time sniffing and biting before either eating or rejecting them. The post-operative changes in affective behaviour were of especial interest. Prior to the temporal pole removal this animal was wild and intractable. When observers looked into its cage the animal would bare its teeth and dash forward, jumping at the door and attempting to bite and claw. Following the temporal pole removal the animal responded placidly to such visual stimulation and would approach the experimenter peacefully and take raisins from his hand. It is important to note that this placid behaviour occurred only in response to visual stimuli. If one attempted to touch the animal or grasp its arm, a full aggressive reaction would occur.

Opening the eye projecting to the undamaged side and closing the other eye resulted in an abrupt change in behaviour back to the pre-operative level. There were no signs of visual agnosia, and the animal showed a persistent aggressive response at the appearance of the experimenter.

The question then arose as to whether or not the change in emotional behaviour to visual stimulation was merely a reflexion of the visual agnosia or due to damage to sub-cortical structures, in particular the amygdaloid complex, which had been removed during the unilateral temporal pole ablation. In an attempt to answer this, another animal (*D*-28) that had previously undergone mid-sagittal sectioning of optic chiasma and cerebral commissures was subjected to unilateral amygdalectomy. To avoid damaging the temporal neocortex, the amygdaloid area was approached from beneath the right frontal pole and removed using the aspiration technique. During the course of surgery, it was found necessary to section the right olfactory tract in order to obtain adequate exposure. Post-operative recovery was uneventful.

This animal showed no signs of visual agnosia irrespective of which eye was open. Similarly, there did not appear to be much change in affective behaviour although, superficially, it seemed as if the animal was a little more placid when the eye ipsilateral to the side of the amygdalectomy alone was open. Another craniotomy was then carried out. The left hemisphere was gently retracted from the falx, and the incision along the length of the corpus callosum was reopened. In addition, the hippocampal, habenular and posterior commissures were completely sectioned. The incision was extended to include the massa intermedia. The supra-optic recess was entered, and the tip of the knife passed between the medial walls of the hypothalamus until the sphenoid bone was reached. Post-operative recovery revealed a transient left abducens nerve palsy and a mild but persistent right hemiparesis.

Following the second operation, this animal did not differ from the typical wild and aggressive monkey when observed with both of its eyes open. At the sight of an observer, it would grimace, bare its teeth and jump around the cage. Closing the eye on the side of the amygdalectomy did not result in any observable change. However, opening this eye and closing the opposite eye produced a dramatic change in behaviour. The animal showed no signs of aggression or fear at the sight of human observers and would approach the front of the cage to take raisins from the experimenter's hand.

As in the instance of the animal with the entire temporal pole removal, this placidity occurred only in response to visual stimulation; touching or prodding the animal's arm would produce a momentary aggressive reaction. The placid response to visual stimuli promptly disappeared when the eyelids on the side of the amygdalectomy were sutured shut and the other eye opened. These alternations in affective behaviour were observed over a period of three months, after which the animal was killed and its brain prepared for histological examination.

Although these observations are of interest in themselves, their primary importance lies in illustrating an approach to the investigation of the behavioural correlates of central nervous system lesions which has the advantage of permitting repeated control and experimental observations of the same animal. In the 'split-brain' animal, a

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unilateral lesion is functionally comparable with a symmetrical bilateral ablation (with respect to visual stimulation) when the eye ipsilateral to the lesion is open and the opposite eyelids sutured shut. When the eye contralateral to the lesion is open and the ipsilateral eyelids closed, the animal behaves with respect to visual stimuli as in its immediate pre-operative state. This suggests that one can, in effect, 'remove' brain tissue and 'replace' it by opening and closing the appropriate eye. Among the several possible extensions of this approach comparing the effects of different lesions performed in opposite hemispheres in the same animal may prove to be especially intriguing.

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INHIBITION OF GROWTH AND PHOTOSYNTHESIS IN SUBMERGED PLANTS WITH TETRAMETHYLTHIURAM DISULPHIDE AND SODIUM DIMETHYLDITHIOCARBAMATE AND REVERSAL OF THIS INHIBITION OF PHOTOSYNTHESIS

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THE biological action of certain dithiocarbamyl compounds has been reviewed by Goksøyr[1]. Their fungicidal action and the inhibition of certain aldehyde dehydrogenases caused by 'Antabuse' account for most of their biological effects. According to Green *et al.*[2] photosynthesis in *Chlorella* is markedly inhibited by sodium diethyldithiocarbamate (Na-DEDT). This effect is related to a similar one of other inhibitors, which like Na-DEDT are denoted "Cu-poisons" by these authors. The inhibition of the oxygen production of isolated chloroplasts by 5×10^{-3} *M* Na-DEDT is about 30–50 per cent[3]. On the other hand, the photosynthetic phosphorylation in isolated chloroplasts is not inhibited by 1×10^{-3} *M* Na-DEDT[4].

No inhibition of photosynthesis by tetramethylthiuram disulphide (TMTD) or homologous substances has been described so far. In a Dutch horticultural paper, however, a gardener reports that he has cleared the soil from algal fouling with TMTD[5].

In the present investigation the action of different concentrations of TMTD and sodium dimethyldithiocarbamate (Na-DMDT) on the growth of 2–5 mm.-long thalli of the green alga *Enteromorpha linza* was investigated using a non-sterile procedure of cultivation. Considerable inhibition of growth was obtained with both substances (Fig. 1). The relatively large dispersion of the replicates results from the inhomogeneity of the inoculated material (cf. controls) and on the instability of the applied chemicals. It is known that the presence of trace elements in the nutrient solution causes the concentration of $DMDT^-$ to drop below the intended level, and that metal-DMDT-complexes, which are possibly toxic, are formed[1]. The number of equivalents necessary in order to induce inhibition seems to be the same for the two substances (Fig. 1).

Starting from the growth inhibitions, caused by TMTD and Na-DMDT, the action of these substances on photosynthesis and respiration in the dark was investigated. Photosynthesis was measured with no correction for the simultaneous light-respiration. The determinations were made using both *p*H measurements and a Warburg-method. Respiration in the dark was determined only with the Warburg method.

The first method involves measurements of the changes in *p*H caused by photosynthesis in submerged plants. Each experiment includes measurements of simultaneous changes in two media, one with and one without inhibitor. When inhibition of photosynthesis occurs, the *p*H is changed to a smaller extent in the solution containing the inhibitor than in the other solution. In this a suitable quantity of algae at adequate temperature and light changes the *p*H from 8 to 9 or more during 2–3 hr. of photosynthesis. Non-protolytes or substances, nearly completely protolysed at *p*H 8, can be tested for inhibitory effect with this method. In this investigation the method has been used to determine the extent of inhibition of photosynthesis by TMTD in different species of submerged plants. The degree of inhibition of photosynthesis is taken from a nomo-

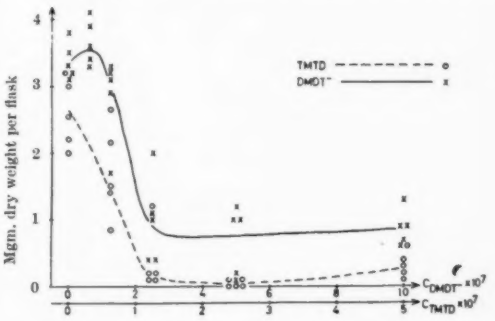

Fig. 1. Growth of thalli of *Enteromorpha linza* at different concentrations (moles per litre) of TMTD and Na-DMDT. Medium: sea water with addition of 0·01 per cent sodium nitrate, 0·005 per cent dipotassium hydrogen phosphate, 0·1 per cent *tris*-buffer, and trace elements chelated with Na_2-versenol (medium *ASP6* (ref. 6)). Initial *p*H 7·6. In each flask 5–10 thalli 2–5 mm. long. Light intensity about 4,000 lux. Temperature 18° C. The TMTD series was gathered after 14 days, and the Na-DMDT series after 13 days. Thalli were dried at 105° C. and weighed

gram which has on a theoretical basis been calculated for each kind of medium applied. Sea water was used as medium for marine algae and fresh water, rich in bicarbonate, for submerged higher plants. The conditions were as follows:

(*A*) Sea water of salinity 32 thousandths. The algae were stored in sea water one day and night before the experiment. The sea water, which was saturated with TMTD, also contained small amounts of DMDT-. The experiments were performed at a light intensity of about 650 lux and at a temperature of about 18° C.

(*B*) Sea water of salinity 25 thousandths. The algae were partly cultivated and partly stored in the medium given in the legend of Fig. 1. The experiment was carried out at an illumination of about 7,000 lux and at a temperature of 18° C. $C_{TMDT} = 0{\cdot}3 \times 10^{-4}$ *M*.

(*C*) Fresh water with an addition of 1 m.mol. sodium bicarbonate and 0·05 m.mol. sodium hydroxide per litre. The plants were acclimatized and cultivated in this medium for 1 and 20 days respectively. The illumination was about 7,000 lux and the temperature 18° C. $C_{TMTD} = 0{\cdot}7 \times 10^{-4}$ *M*.

About equal quantities of plants were placed in four test tubes containing 10 ml. solution, two of the tubes with TMTD and two without inhibitor. No corrections have been introduced in order to compensate for the differences in the photosynthesis before the addition of the TMTD solution.

The extent of inhibition (Table 1) is given in per cent of photosynthesis without inhibitor.

Table 1

	Condition	Inhibition (per cent)
Chlorophyceae		
Ulva lactuca	*B*	70, 85
Enteromorpha compressa	*A*	100, 105
Enteromorpha linza (not cultured)	*B*	85, 105
Enteromorpha linza (cultured)	*B*	110, 115
Cladophora rupestris	*A*	70, 80
Acrosiphonia centralis	*B*	30, 50
Phaeophyceae		
Pylaiella littoralis (cultured)	*B*	60, 75
Ectocarpus confervoides	*A*	80, 80
Rhodophyceae		
Ceramium strictum	*A*	100, 100
Ceramium rubrum	*A*	95, 100
Callithamnion corymbosum	*A*	90, 100
Polysiphonia urceolata	*B*	45, 50
Submerged moss		
Campylium sp.	*C*	70, 80
Submerged higher plants		
Utricularia intermedia	*C*	40, 55
Hydrilla verticillata	*C*	95, 105

According to this the photosynthesis of submerged plants seems generally to be inhibited by TMTD.

The concentrations of TMTD in the water solutions used have been analysed according to a method worked out by Åkerström and based on some of his investigations[7]. Work by Åkerström and me on the effect of derivatives of TMTD and of related substances on photosynthesis is progressing.

Enteromorpha linza used in the experiments with the Warburg method has been taken partly from a non-sterile culture, *E.l.*(cult), in the nutrient solution (cf. legend to Fig. 1) and partly from the sea, *E.l.*(sea), off the west coast of Sweden. The *E.l.*(sea), before being used, was kept in light in the nutrient solution for different lengths of time.

The manometric experiments have been carried out in conical vessels of about 17 ml. at 18° C. and with a light intensity of 6,800 lux at the bottom of the vessel. The bicarbonate solution described in the legend of Fig. 1 was used, but without addition of Na_2-versenol and trace elements. Its *p*H was adjusted to 8·3–8·4, after which the solution was brought to equilibrium with atmospheric carbon dioxide by aeration, and 3·0 ml. of this medium with or without inhibitor was pipetted into the main compartment and 0·5 ml. into each side-arm. Readings were taken every 5 min. for 100–140 min. The quantity of algae in each vessel was chosen so as to make the increase in pressure not to exceed 70 mm. Brodie solution.

In order to measure a possible inhibition of endogenous respiration experiments were performed in the dark. These differed from the above only as regards the duration of the experiments and the illumination. Because of the low respiratory-rate, readings were taken every 30 min. for 280–340 min. The quantity of algae in each vessel was chosen so as to produce a decrease in pressure not less than 25 mm. Brodie solution. The ratio of the oxygen production in light and the oxygen uptake in the dark of the same thallus varies from 35/1 to 25/1. From this it is obvious that the inhibition of the photosynthesis studied cannot be interpreted as a consequence of stimulation of respiration.

TMTD and Na-DMDT inhibit photosynthesis in both *E.l.*(cult) and *E.l.*(sea). Fig. 2 shows the extent of this inhibition in *E.l.*(cult) at different concentrations of both substances. TMTD has evidently a much stronger effect than Na-DMDT. A comparison between Figs. 1 and 2 reveals that the ranges of concentration in which the inhibition of photosynthesis occurs do not coincide with the corresponding range of concentration for inhibition of growth. In the dark no inhibition of the endogenous respiration in *E.l.*(cult) is obtained either by $0{\cdot}38 \times 10^{-5}$ *M* TMTD or by 1×10^{-4} *M* Na-DMDT.

The inhibition of photosynthesis by 1×10^{-4} *M* Na-DMDT in *E.l.*(sea) can be lowered by addition of equivalent quantities of certain metal ions, namely, Cd^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} and Au^{3+}. In some experiments with Cu^{2+} complete reversions of the inhibitions were obtained. No reduction of the inhibition is obtained by the same concentration of Mn^{2+}, Sn^{2+} and Fe^{2+}, while at the same concentration of Zn^{2+} photosynthesis is further diminished. Besides, the inhibition is decreased by 1×10^{-4} *M* glutathione and 2×10^{-4} *M* cysteine, whereas 1×10^{-4} *M* of the latter substance does not reduce the inhibition.

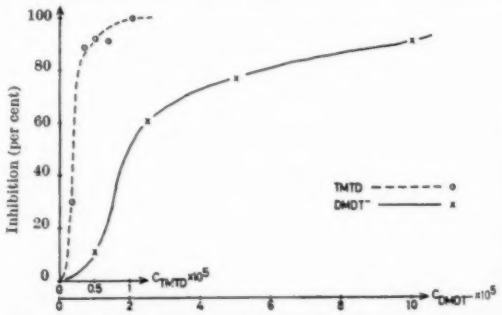

Fig. 2. Inhibition of photosynthesis in *Enteromorpha linza* (cultured) at different concentrations (moles per litre) of TMTD and Na-DMDT

In a corresponding manner, an inhibition induced by TMTD has been investigated. The inhibition in *E.l.*(cult) by $0{\cdot}69 \times 10^{-5}$ *M* TMTD is not lowered by addition of 1×10^{-4} *M* Cu^{2+}, whereas this is the case in the presence of 1×10^{-4} *M* Au^{3+}. The inhibition of photosynthesis in *E.l.*(sea) by $0{\cdot}54 \times 10^{-4}$ *M* TMTD and in *E.l.*(cult) by $0{\cdot}36 \times 10^{-4}$ *M* TMTD is considerably diminished by the addition of 1×10^{-4} *M* glutathione, and in the former material also by 2×10^{-4} *M* cysteine.

It appears from the above that the inhibition of photosynthesis caused by both TMTD and Na-DMDT is almost completely reversible. The reduction of the Na-DMDT-induced inhibition by different metal ions can be explained by the drop in concentration of $DMDT^-$, caused by the formation of metal-$DMDT^-$ complexes. The fact that the TMTD-induced inhibition is not diminished by addition of 1×10^{-4} *M* Cu^{2+} could, however, be due to the circumstance that $DMDT^-$ ions *per se* are not responsible for this inhibition. Under the presumption that the photosynthesizing plant is able both to oxidize $DMDT^-$ and to reduce TMTD, the observed phenomena may be regarded as effected by TMTD only. In keeping with this is the fact that addition of glutathione or cysteine diminishes inhibition caused both by Na-DMDT and TMTD. Further, the reversal of the inhibition with metal ions, when this is induced by $DMDT^-$, could thus be due to the ability of the plant to reduce earlier formed TMTD to $DMDT^-$, which is then complexed.

A full description of the present investigations will appear in *Svensk Botanisk Tidskrift* and *Physiologia Plantarum*.

I am indebted to Prof. N. Fries, Dr. K.-G. Nyholm, Prof. P. E. Lindahl, and Dr. S. Åkerström for their interest and help with this work.

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A PHYTOPLANKTON MULTI-SAMPLER AND ITS USE IN LAKE VICTORIA

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FROM April until August 1960, many phytoplankton samples were taken with a Ruttner sampler from the northern end of Lake Victoria, especially in the north-west corner of Pilkington Bay of Buvuma Island (Fig. 1). The main constituents of the phytoplankton were *Melosira nyassensis* var. *victoriae*, *M. granulata*, *M. varians*, *M. agassizii*, *Aphanocapsa elachista*, *Lyngbya circumcreta*, *Anabaena circinalis* and *Merismopedia elegans*. Certain other diatoms and blue-green algae were observed sporadically as were twelve species of the Chlorococcales. Very wide variations in the horizontal and vertical distributions of phytoplankton were found in Pilkington Bay, and in the north-west corner of the bay there was a sharp overall increase in phytoplankton during May. The variations in distribution, and the May increase, although the latter might have been a reflexion of seasonal change, were possibly due to water movements. The possible association between water movements and variations in plankton density has been mentioned by Talling[1], and Fish[2] has referred to exchanges between deep and shallow water and the possible increase in fertility of the main lake resulting from the outward movement of fertile inshore waters.

To investigate water movements and the carriage of phytoplankton near the surface, a multi-sampler was designed and constructed (Fig. 2). This consisted of a 'Dexion' No. 112 alloy-angle framework with four empty, sealed 1-gallon cans acting as floats. Eight phytoplankton nets held on galvanized steel loops were attached to the frame. The narrow end of each net was tied tightly on to a connecting tube of plastic, which in turn led into a collecting tube which, kept in place only by a close-fitting rubber sleeve, could

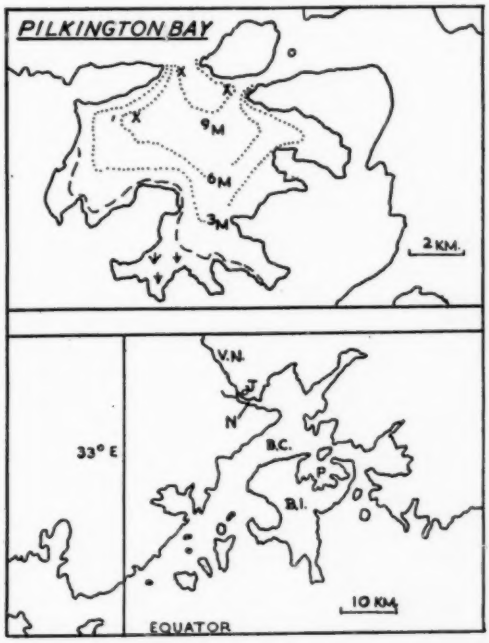

Fig. 1. Pilkington Bay with depth contours in metres; *x*, approximate positions that phytoplankton multi-sampler was used. Lower map shows the position of the bay (*P*) in Buvuma Island (*B.I.*) at the northern end of Lake Victoria. *V.N.*, Victoria Nile; *J*, Jinja; *N*, Napoleon Gulf; *B.C.*, Buvuma Channel

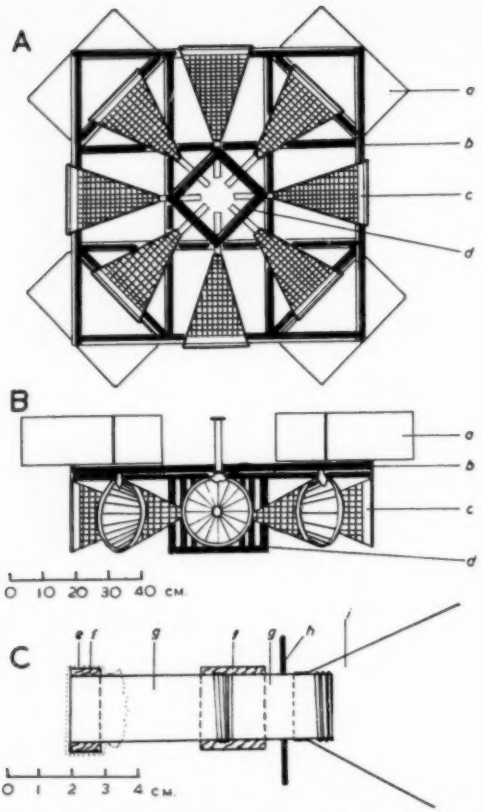

Fig. 2. The phytoplankton multi-sampler. *A*, plan view, from beneath; *B*, elevation; *C*, collecting tube in half-section. *a*, Float; *b*, framework of 'Dexion' No. 112 alloy-angle; *c*, phytoplankton net on wire loop; *d*, centre-piece of framework; *e*, fine-mesh phytoplankton netting; *f*, rubber tubing as sleeve; *g*, plastic tube; *h*, edge of alloy angle upright of centre-piece; *i*, inner end of phytoplankton net

easily be detached. Each collecting tube was closed at its outer end (away from the net) by a piece of phytoplankton mesh retained over a short length of rubber sleeving by a rubber band. Wide-mouth, screw-cap, 9 × 5 cm. plastic bottles, each containing about 10 ml. sterile, distilled water and each marked *N*, *N.W.*, *W*, etc., were carried for storing the collecting tubes.

The multi-sampler was placed in the water with the centre of each net opening at about 15 cm. below the surface. With the floats marked *N*, *S*, *E* and *W*, the multi-sampler was orientated in a north–south direction and held in position from a small launch or from a dinghy at anchor, usually for 10 min. At the end of this time the collecting tubes were detached carefully and each placed quickly in the appropriate bottle. In the laboratory, the contents of each collecting tube were washed into a 50 ml. measuring cylinder, made up to a known volume and, after shaking, a sample taken for a quantitative determination of the phytoplankton by the sedimentation technique[3,4]. The results, after corrections for dilutions and the time of each run, were recorded for each net as units/min. All species of *Melosira* were counted together as cells, species of *Pediastrum* and *Scenedesmus* as cœnobia, *Anabaena circinalis* as cells, *Lyngbya circumcreta* as coil turns, *L. limnetica* as filaments, *Aphanocapsa elachista* as colonies, *Merismopedia elegans* as groups of eight cells and all other algae as individual cells. During each use of the multi-sampler, three surface-water samples were taken to determine the general concentration of phytoplankton in the vicinity of the apparatus.

The first trial run was in the Napoleon Gulf where there was known to be a current of about 20 cm./sec. In parallel with the multi-sampler, an Ekman current meter was used. The multi-sampler results can be plotted as histograms and vector diagrams, and four of them are shown in Fig. 3. The resultant component indicates the general flow direction during the sampling period, and this was found to be close to that given by the Ekman current meter (Fig. 3*A*). The resultant component can also be regarded as being approximately related to the rate of flow.

As there are practical difficulties involved, such as local turbulences, net resistances at higher rates of flow and net clogging in high concentrations of phytoplankton, it is not proposed here to attempt to calculate accurately absolute velocities of currents from the multi-sampler results. It is believed, however, that the multi-sampler results, in comparison with the phytoplankton concentrations in the water samples taken in parallel, do give some indication of current velocities and a comparison of several such results with actual rates of flow as determined with the Ekman current meter (Table 1) supports this. For the sake of simplicity, however, the result for a single net, that which collected the most phytoplankton, has been used, rather than the resultant component of the vector diagram, in calculating the current velocity. This method, although possibly less precise, is believed to be sufficiently accurate for such approximations. In two of the results included in Table 1, only counts of *Melosira* were considered as all the blue-green algae on those occasions were restricted to the water surface. These results suggest that provided there are no serious turbulences there is a relationship between current velocity and the quantity of phytoplankton trapped by the net most closely facing the prevailing current.

That is:

$$F = P.k \text{ cm./sec.}$$

where F = calculated rate of flow;

Table 1. Relationships between Rates of Flow as determined by Ekman Current Meter and from Phytoplankton Counts

Time and place	E = rate of flow in cm./sec. measured from an Ekman current meter	P = phytoplankton units/min. / phytoplankton units/ml.	$k = E/P$
(1) Napoleon Gulf 1600–1610 30.5.60	19.5	13	1·50
(2) Pilkington Bay 1420–1430 30.8.60	< 6·0	4	< 1·50
(3) Pilkington Bay 1730–1740 24.6.60	5·0	4	1·25

For (1) and (3) only *Melosira* counts, as cells/min. and cells/ml. were included in the calculations as, on these occasions, the Cyanophyceae were restricted to the water surface above the level of the nets.

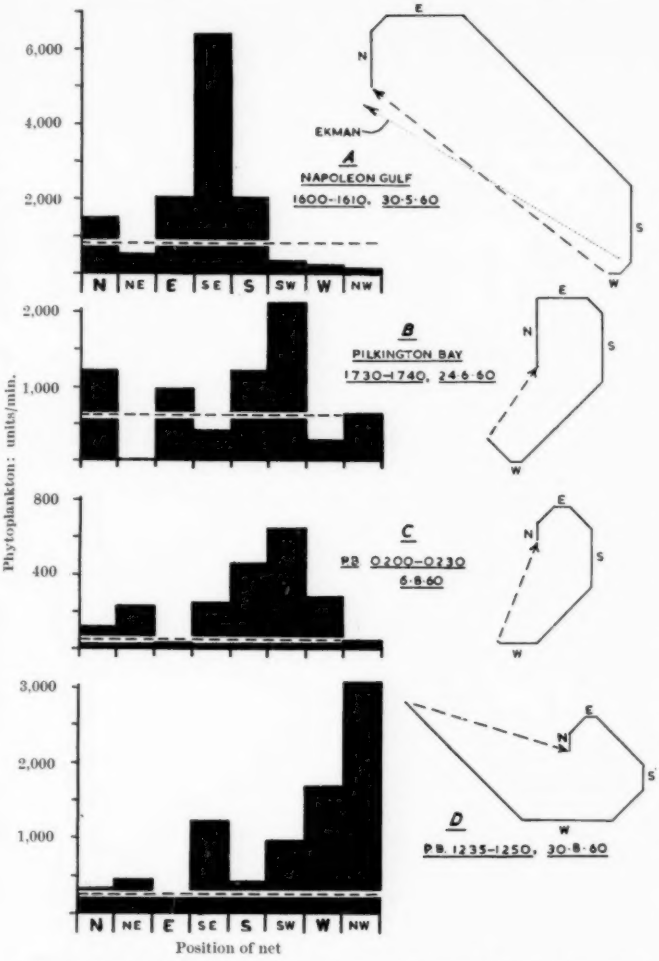

Fig. 3. Some results obtained with the phytoplankton multi-sampler. For the histograms, the broken horizontal line represents the mean density of phytoplankton, in units/ml., in the surface water during the sampling period. For the vector diagrams, on the right, the results have been plotted in the eight directions of the compass, but the diagrams are not all to the same scale. The broken line of the vector diagrams is the resultant component, and indicates the general flow direction. For the top figure (*A*), the flow direction indicated by an Ekman current meter is included for comparison. For *D*, the multi-sampler was at the mouth of the bay

$$P = \frac{\text{phytoplankton units/min.}}{\text{phytoplankton units/ml.}} \text{ in water sample ;}$$

k is a value in the range 1·25–1·50.

As is to be expected, with decreasing velocities there was a closer and closer approach of calculated rate of flow to actual rate of flow. Below a rate of flow of about 5 cm./sec. the Ekman current meter will not register over a short period of time. The multi-sampler, however, will give results, within 10 min., at rates of flow of less than 5 cm./sec. These results may be used comparatively, and, bearing in mind that they are still approximations, some indication of absolute rate of flow may be determined. In one series of samples, for example, the calculated rate of flow was in the range 1·1–1·3 cm./sec., and it is not unreasonable to conclude that the actual rate was in the range 1·0–1·5 cm./sec.

The area under investigation is well removed from the Lake Victoria outflow, and the major cause of surface water currents was the wind (cf. Hutchinson[5]) which, until about midday on most days, tended to blow gently into the Bay from a northerly direction and then change abruptly to a stronger wind from a southerly direction. The results for Fig. 3*D* were obtained, however, at a time when a strong wind from west-north-west produced a surface flow of about 15 cm./sec. Surface water currents from, or near, the south-west, south or south-east would tend to carry phytoplankton from shallow inshore regions to the mouth of the Bay and through into the open, deeper water of the Buvuma Channel and so, possibly, act as an inoculum of algae for the open water of the lake north of Buvuma Island. At the same time there would be an inflow of water, below the surface, into the Bay, which might result ultimately in the return of algae, such as *Melosira*, sunk deep in the Buvuma Channel, to the photic zones in the shallower, inshore parts of the Bay. An incoming, oblique up-current was once observed, by virtue of a dense concentration of visible clumps of blue-green algae, from a dinghy anchored in the mouth of the Bay. This current was possibly due to a combination of a wind from the north and a local internal *seiche* movement.

The generally slower surface currents from northerly directions carried some algae into the Bay, and during one sampling (Fig. 3*D*) about 95 per cent of the phytoplankton from the north-west was *Melosira*. The current veered round completely during the sampling period, however, and it was found that currents from the south and south-east, and from the north-east, contained 70–90 per cent Cyanophyceae. This suggests the occurrence of localized areas of *Melosira* outside the Bay to the north-west and of Cyanophyceae to the north-east and inside the Bay.

A near replica of the phytoplankton multi-sampler has been constructed for trials in Britain, and an entirely new, more compact multi-sampler is being devised for later use.

My thanks are due to the Colonial Office for the award of a research fellowship, and to the staff of the East African Fisheries Research Organization for much practical help and encouragement during this and other work carried out in Central East Africa.

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LETTERS TO THE EDITORS

RADIOASTRONOMY

Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts

In the radio-frequency spectrum, solar radio bursts have been classified according to their dynamic structure. Information on the velocity of shock waves or the solar corpuscular stream as well as on the physical mechanism of the radiation has been obtained by this recording method. Another technique for studying the mechanism of solar radio radiation is to observe the modulation structure of bursts and the quiet Sun at discrete frequencies. Jager and Veer[1] studied solar radio transients by recording 200 Mc./s. radio noise on fast paper tape. Their observations were concerned with the time duration and distribution of radio-pips, their bandwidth and the presence of the echo phenomena.

In an effort to study the very-low-frequency details of different types of solar radio bursts, observations were made at the Sagamore Hill Radio Observatory, Massachusetts, on three discrete frequencies, 220 Mc., 400 Mc. and 3,000 Mc./s., with an 84-ft parabolic antenna. The magnetic tape records, taken to observe d.c. to 600 c./s. fluctuations, were analysed over 5–550 c./s. to detect enhancements or absorption in this range. The power spectra of the modulation envelope show that the high-frequency end of the very-low-frequency band is accentuated.

Type II and type IV bursts reveal marked attenuation over certain very-low-frequency bands. An isolated burst at 220 Mc./s. was associated with a sharp and fast development on one particular band.

The spectra of random noise, limited to a narrow intermediate-frequency band of centre frequency f_0, may be written as:

$$x(t) = x_i(t)\cos 2\pi f_0 t + x_q(t)\sin 2\pi f_0 t \qquad (1)$$

where $x_i(t)$, $x_q(t)$ are in-phase and quadrature components respectively at any instant and are totally independent of each other.

Assuming square-law detection, it can be shown that the power spectra of the envelope of narrow band-limited random noise will be triangular. The triangular spectrum when sampled over a very small band compared with the band limit of the noise will yield a flat spectrum. In the present investigation the very-low-frequency band extended from 5 to 550 c./s., which is very small compared with the intermediate-frequency band-widths of at least 1 Mc./s. In this case, therefore, the modulation spectrum of the random noise in the envelope is expected to be flat.

Solar radio radiation was received at 220 Mc., 400 Mc. and 3,000 Mc./s. The intermediate-frequency band-widths varied, ranging from 1 Mc. at 220 Mc. to 200 Mc. at 3,000 Mc./s. (using travelling-wave tubes in the latter system). The intermediate-frequency signal, approximately a square bandpass, was detected in square-law detectors and recorded on a frequency-modulated magnetic tape. The solar event of August 11, 1960, provided an opportunity to record all types of bursts. Using the dynamic spectra taken at Fort Davis, Texas, by A. Maxwell and A. R. Thompson (private communication), particular periods of type II, type III and type IV bursts could be selected and the very-low-frequency spectra analysed within the range of 5–550 c./s. The records were normalized against noise tube and noise diode spectra.

Theoretically, the spectrum of the noise generator envelope within the very-low-frequency band was expected to be flat. Fig. 1, showing this spectrum, illustrates the relatively flat frequency response of the entire receiver, recorder, and analysing system. The spectrum of quiet Sun emission is also observed to be flat, as illustrated in Fig. 2.

However, the envelope spectra of all types of bursts when normalized against noise samples show definite structure (Fig. 3, *a–d*). The enhanced response at 60 c./s. and its harmonics may be disregarded as possible power frequency pick-up. The high end of the band is accentuated in all three frequency channels. Type II and type IV burst envelopes display certain bands with marked attenuation (Fig. 3, *a–d*). These spectral regions are distributed throughout the analysed band and have a maximum band-width of approximately 60 c./s. The spectrum of the envelope of an isolated burst shows sharp and fast development of structure around two narrow very-low-frequency bands (arrows in Fig. 4). This is followed by a gradual widening of the feature. The time-interval between succeeding spectra is 4 sec.

The emphasis on the high-frequency end of the very-low-frequency power spectra of the envelope indicates that the solar noise bursts do not fit the

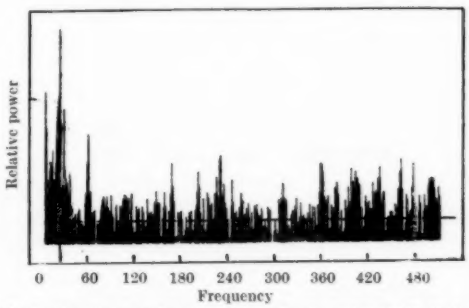

Fig. 1. Envelope spectra of random noise generator at 220 Mc./s. The dashed line shows the average power spectra. Enhanced response at 60 c./s. and 120 c./s. is due to power frequency pick-up

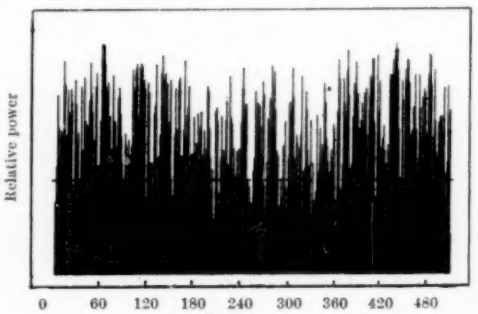

Fig. 2. Envelope spectra of radio noise from the quiet Sun at 220 Mc./s. on March 10, 1961, at 1756 U.T.

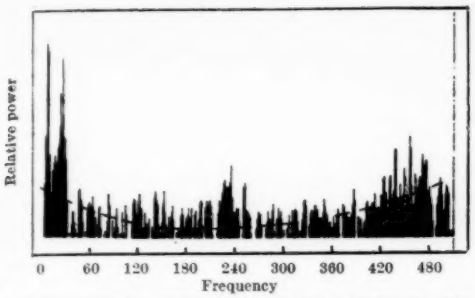

Fig. 3*a*. Envelope spectra of slow drift burst from the Sun at 220 Mc./s. on August 11, 1960, at 1929 U.T.

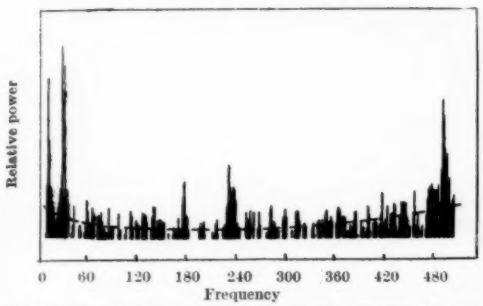

Fig. 3*b*. Envelope spectra of continuum emission from the Sun at 220 Mc./s. on August 11, 1960, at 2003 U.T.

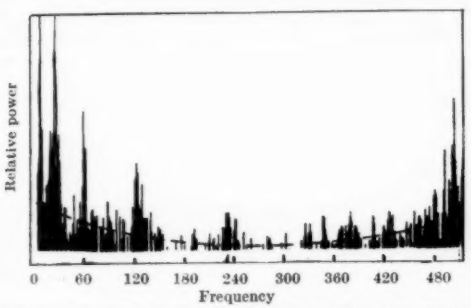

Fig. 3*c*. Envelope spectra of continuum emission from the Sun at 400 Mc./s. on August 11, 1960, at 2003 U.T.

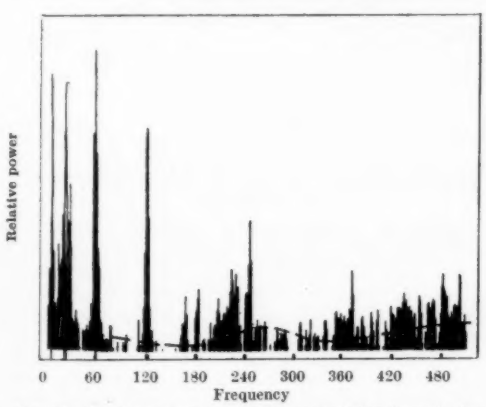

Fig. 3*d*. Envelope spectra of continuum emission from the Sun at 3,000 Mc./s. on August 11, 1960, at 2003 U.T.

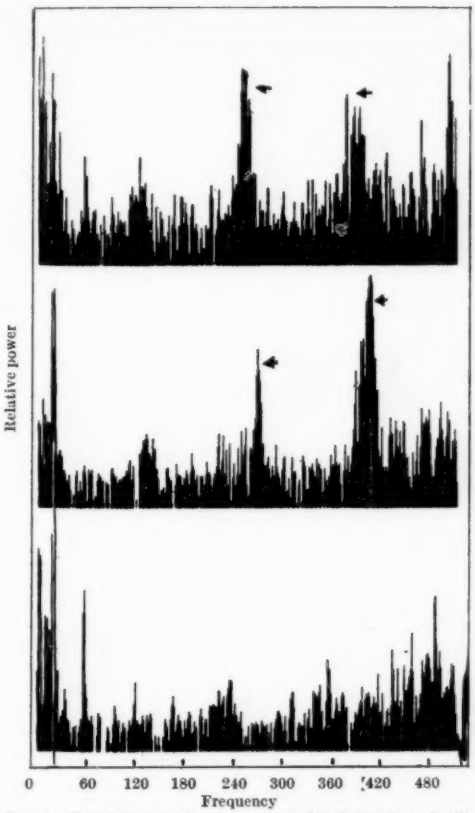

Fig. 4. Development of a fast narrow-band structure in the envelope spectra of an isolated burst from the Sun at 400 Mc./s. on August 11, 1960, at 2052 U.T.

normal law of random functions. The development of a narrow-band structure in the case of an isolated burst may be explained on the basis of a model in which the source is situated above the zero-refractive-index layer and a delayed interaction exists between the direct and the reflected ray. The consequent phase delay might give rise to a definite very-low-frequency modulation structure. A proper distance scale between the source and the reflector might account for the attenuation bands in type II and type IV spectra. The possibility of an inherent modulation at the source from the shock wave mechanism also exists.

A more detailed study of the modulation spectra over a wider band should be undertaken before any hypothesis is advanced on the envelope spectra of different types of solar bursts. A new technique for observing solar mechanisms might result, particularly if the effect of the magnetic field around sunspots as well as a general magnetic field could be observed by the envelope studies of the modulation process.

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Lunar Occultation of a Radio Source

At present only a small number of radio sources have been satisfactorily identified with photographic objects. The important task of establishing further identifications faces a serious difficulty because of the uncertainty in the measured positions of the radio sources. This uncertainty usually amounts to several minutes of arc, and it is only for a few intense sources that the error has been reduced to less than one minute of arc.

It has been suggested by several authors, for example by Link and Neuzil[1], that observations should be made of the occultation of radio sources by the Moon, and this technique has already been used to study some intense sources of large angular diameter[2–5]. It is the purpose of this communication to direct attention to the high precision with which source positions can be measured by observing lunar occultations with equipment which can track the Moon continuously. The results of a preliminary test of the technique on a radio source are described here.

On December 8, 1960, the 250-ft. radio telescope at Jodrell Bank was used for observing the occultation by the Moon of the radio source 3*C* 212 (ref. 6). The measurements were made at a frequency of 237 Mc./s. using a receiver with a band-width of 2 Mc./s. and a post-detector time constant of 4 sec. The observations were carried out by recording the output of the receiver with the telescope in automatic motion following the centre of the Moon. The beam-width of the aerial was about 1·2° between half-power points and therefore covered an area considerably larger than the Moon's disk.

The occultation was observed as a sharp fall in the received power as the source was covered by the Moon, followed later by a sharp rise as the source reappeared. The measured times at which these events occurred are:

Time of disappearance	08h. 03m. 52·5s. ± 5s. U.T.
Time of reappearance	08h. 40m. 49s. ± 5s.

These times correspond to the passage of the source across the limb of the Moon and they have been derived from the records on the assumption that the angular size of the source is small compared with the diffraction pattern of the Moon's limb.

H.M. Nautical Almanac Office has kindly calculated the celestial co-ordinates of the source from the observed times which define two possible positions of the source, one on either side of the centre of the Moon on a line perpendicular to its direction of motion. In principle this ambiguity can be resolved by observations of a subsequent occultation; however, in the present case, the position given in the Cambridge 3*C* Catalogue is sufficiently precise to allow one of the two possible positions to be chosen. The calculated mean place of the source for 1950·0 is given below together with the data from the Cambridge 3*C* Catalogue.

	Position computed from occultation (1950.0)	
Source 3C 212:	*R.A.*	08h. 55m. 55·7s. ± 0·3s.
	Dec.	14° 21′ 27″ ± 2″
	Catalogue data (6)	
Source 3C 212:	*R.A.*	08h. 56m. 00s. ± 5s.
	Dec.	14° 25′ ± 8′
	Flux	21×10^{-26} W. m.$^{-2}$ (c./s.)$^{-1}$ at 160 Mc./s.

The quoted errors in the computed position have been estimated only from the errors in measurement of the time of immersion and emersion, and neglect any possible refraction in a lunar ionosphere or the effect of irregularities in the Moon's limb. The high degree of accuracy achieved is due to the circumstances of the occultation. The closest approach of the source to the centre of the Moon was about 12 minutes of arc, and under these conditions an error of one minute in the duration of the occultation introduces an error of only 12″ of arc in the declination. If the source had passed close to the centre of the Moon the accuracy would have been considerably reduced, as in this case a similar error in duration would have produced a declination error of about three minutes of arc.

The measurement reported here probably represents the most accurate determination yet made of the position of a radio source. The method used has the great advantage that it is not subject to uncertainties introduced by refraction in the Earth's troposphere or ionosphere. It is also free from the effects of confusion, as the solid angle subtended by the Moon's diffraction pattern is only of the order 1/100 sq. degree. A significant source of error is likely to lie in the assumption that the angular diameter of the source is negligible. However, a preliminary comparison of the observed occultation curves with the theoretical diffraction pattern shows that the diameter of the source must be less than or equal to eight seconds of arc, and hence the resultant error in declination will not be greater than approximately four seconds of arc. It may therefore be concluded that, taking into account all sources of error, the estimated position of the source is probably accurate to within about five seconds of arc.

The work described here was carried out as part of a programme designed to measure accurate positions of a number of radio sources by the method of lunar occultations. A detailed description of the method together with an analysis of all the data so far obtained will be published later.

I wish to thank Sir Bernard Lovell and Prof. R. Hanbury Brown for their interest in the work, and Mrs. Flora McBain Sadler of H.M. Nautical Almanac Office for the calculation of the position of the source.

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PHYSICS

Thermoelectric Power of Single Crystals of Gallium

As part of a programme to investigate the anisotropy of non-cubic crystals, the thermoelectric power of single crystals of the orthorhombic metal gallium has been examined. The aim of the programme is the explanation of the large magnitude of the anisotropies of the physical properties of gallium.

The single crystals were prepared from gallium of at least 99·99 per cent purity, and they were grown in the form of cylindrical rods encased in a glass tube. The length of the specimens was 10–13 cm. and their

diameter was about 0·35 cm. The single crystals were grown from seed by passing a freezing plane through the super-cooled melt and three crystals were produced such that the rod axis in each case was aligned parallel to a principal crystallographic axis. In each case the rod axis and crystallographic axis coincided to one degree or better.

The three crystals were set up so that their upper ends protruded into a 'Perspex' container. Commercial copper wire, 20 s.w.g., was used as the reference material after being annealed to cherry red heat. A copper lead was welded to each end of the crystal to give a good mechanical and electrical contact. During this procedure, care was taken to minimize constraints in the annealed copper. The three sets of leads were connected to the three channels of a Cambridge vernier potentiometer which was used to measure the Seebeck e.m.f.'s. A Hilger-Watts *F.A.*1 galvanometer-amplifier was used as a null detector for the potentiometer, which was graduated in 0·1-μV. steps. By galvanometer interpolation it was found possible to estimate potentials to 0·02 μV.

The lower ends of the specimens were immersed in a Dewar flask of alcohol. The upper 'Perspex' container was filled with a mixture of ice and water and the temperature of the lower bath altered by the addition of solid carbon dioxide, the temperature being recorded by an alcohol-in-glass thermometer. In this manner the Seebeck e.m.f. (e) of the three crystals with respect to copper was measured with one junction maintained at 0° C., while the lower junction was maintained at temperatures + 30° C. to −70° C.

Fig. 1 shows the results obtained for the three crystals aligned along the (100), (010) and (001) crystallographic axes. With the resources available, it was not found possible entirely to eliminate spurious thermal e.m.f.'s set up in the measuring circuit, but the results near the origin of Fig. 1 indicate that these were fairly small compared with the magnitudes measured. The normal convention as regards the sign of the Seebeck e.m.f.'s was used, that is, the e.m.f. is considered positive if the electrical current produced by the temperature gradient flows from the variable to the ice-cold junction through the specimen.

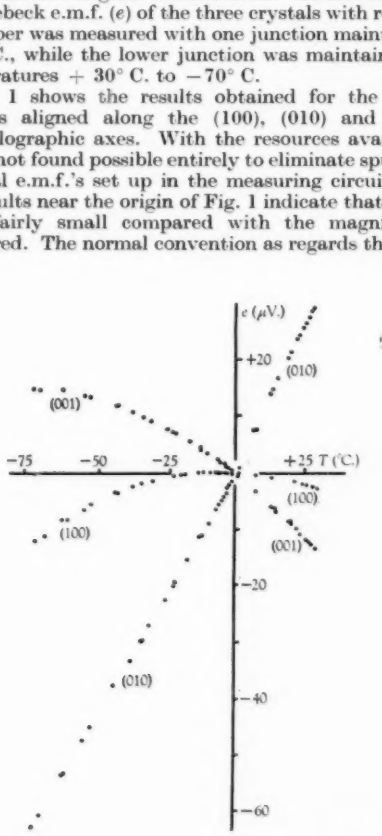

Fig. 1. The variation of the Seebeck e.m.f. (μV.) with temperature of the variable junction (° C.) for three gallium crystals aligned along the principal axes

Table 1 gives, with respect to copper, details of the thermoelectric power (Q) of the specimens as calculated from Fig. 1, that is, $Q = (de/dT)$. As is usual, the variation of Q with temperature is approximately linear, being of the form $Q = A + BT$. The respective values of the coefficients A and B are shown in Table 1.

Table 1. Gallium Single Crystals—Thermoelectric Parameters with Respect to Copper

Crystal orientation	(100)	(010)	(001)
Thermoelectric power (Q) at 0° C. in μV. deg.$^{-1}$ C. (A)	$-0{\cdot}02_3$	$+0{\cdot}96_2$	$-0{\cdot}38_9$
Temperature coefficient of Q in μV. deg.$^{-2}$ C. (B)	$-0{\cdot}005_3$	$+0{\cdot}003_8$	$-0{\cdot}004_7$

Though the scale of Fig. 1 is too small to show the details in the region of the origin, the results for the (100) crystal exhibit, with respect to copper, a neutral temperature at about − 3° C. and an inversion temperature at about − 6° C.

The absolute thermoelectric power of copper has been determined by several workers and the latest results[1] give good agreement with previous values. These authors found that the absolute thermoelectric power (Q^*) of copper was not significantly affected by impurities above 100° K., and that it could be represented by the expression:

$$Q^*_{Cu} = +5{\cdot}4 \times 10^{-3}\,\theta$$

where θ is in degrees absolute. Using this information, the present results can be corrected to absolute values. Again, expressing the results in the form $Q = A + BT$, where T is in degrees centigrade, this gives:

$$Q^*_{Ga(100)} = 1{\cdot}46 + 0{\cdot}000_2 T$$
$$Q^*_{Ga(010)} = 2{\cdot}44 + 0{\cdot}009_2 T$$
$$Q^*_{Ga(001)} = 1{\cdot}10 + 0{\cdot}000_7 T$$

These results are interesting in that the anisotropy ratios of the coefficients A are not as large as those of the electrical or thermal conductivity[2]. The B coefficients are also interesting in that the coefficient for the (010) axis is considerably larger than those for the (100) and (001) axes. For the latter axes, the value of B is practically zero, which indicates that the Thomson coefficients ($T(dQ^*/dT)$) for these two directions are practically zero for the range of temperature under consideration. It is considered that these effects may be of fundamental importance and it is hoped to discuss them more fully later.

This programme on anisotropy is under the general direction of Prof. A. J. Woodall, to whom thanks are due for encouragement and advice. The Dean of the Royal Military College of Science is thanked for permission to publish this communication.

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XUM

Neutron Terminology

Weinberg and Wigner[1] have made a plea for a more scholarly exposition of reactor technology; Ghose and Young[2] suggest that the proposed introduction of a unit of neutron flux be made an opportunity to make customary the use of the word 'flux' in this context. Their suggestion, however, involves an over-simplification, through neglecting to make allowance for the two concepts of neutron current and neutron flux, the importance of which I have emphasized elsewhere[3].

In reactor terminology, neutrons traversing unit area in unit time are measured as neutron current, not as neutron flux. It would be more correct to say neutron current density, but there is no good reason to prefer flux density. Neutron flux has, conventionally, the same dimensions as neutron current, but arises from a different point of view: whereas neutron current is a rate of crossing a surface by neutrons, neutron flux is a rate of track formation by the neutrons in a volume, being equal to the neutron density (in the sense of concentration) times the mean speed. If Ghose and Young's suggestion of calling this quantity 'flux density' were adopted, then flux would become total number of neutrons times mean speed—a change that would make confusion worse confounded. It should be noted that though a unit of flux would, on dimensional considerations alone, be valid for neutron current also, this extension of its use would lead to a possibility of error because, in going from flux to current, a factor of $\frac{1}{2}$ appears (for random motion) as a result of the inclination of the line of flight to a surface becoming involved.

While feeling that the meaning at present attached to 'neutron flux' can be justified, there is another usage which cannot. Whereas a microscopic cross-section has dimensions L^2, the so-called macroscopic cross-section has dimensions L^{-1}: it is not a cross-section at all. It can, for example, be an absorption coefficient, and has the correct dimensions for one. Terms such as 'absorption coefficient' are already well known, are less cumbersome, and using them instead of macroscopic cross-section would permit the word 'microscopic' to be dropped when referring to a true cross-section.

It would, furthermore, lead to greater clarity if 'multiplication constant' were reserved for a regular lattice of fuel and moderator of infinite extent, and not used for the most important variable in a real reactor. For the latter, 'reproduction ratio' seems the appropriate choice.

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Neutron 'Flux'

It is the title, not the definition, of 'neutron flux' which is ambiguous, particularly the application of a vector term to a scalar quantity. The title should imply some connexion with the flow of neutrons; in fact, as the velocities are integrated regardless of direction, the function defines the rate of generation of neutron track-lengths per unit volume at a given point. This is important in reaction problems because, as the elementary particles are treated as spherical and hence presenting a fixed cross-section to any neutron, it measures the collision potential. It is nothing whatever to do with the flow of neutrons, except in such special cases as collimated beams or by indirect calculation when the distribution pattern has been determined; yet the phrase gives this impression and misleads many entrants to the nuclear field.

The mere fact that neutron flux, like the pressure or temperature of a gas, is caused by the movement of the particles involved, is no good reason for applying to a scalar a title which in all other branches of science is reserved for vectors; my own preferred alternative is neutron (collision) potential, but even a synthetic proposal would be better than the present term.

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CRYSTALLOGRAPHY

'Effective' Ionic Charge in Crystals

A recent communication by Mooser and Pearson[1] has directed attention to the confusion which can result from two distinct usages of the term 'ionic'. As an example they cite discussions of the bonding in zinc blende, where the tetrahedral bonds may be said to arise from resonance involving (i) $Zn^{2-}S^{2+}$ and (ii) $Zn^{2+}S^{2-}$. The first is the extreme covalent structure which gives each atom four valence electrons; the second is conventionally called the fully ionic structure since both atoms have closed-shell configurations. As Mooser and Pearson point out, both (i) and (ii) are physically ionic, and the convention which describes 'neutral bonded' Zn^0S^0 as 50 per cent ionic, because it involves equal resonance contributions from (i) and (ii), can be most misleading.

The question of the ionic charge in compounds isomorphous with zinc blende, a class which includes several important semi-conductors, has been much discussed, since for such purposes as energy-band calculations or the calculation of electronic levels at a vacancy it is necessary to know the electrostatic potential in the crystal. There is considerable confusion in the literature on the question of how this ionic charge may be measured. The confusion is due partly to the situation to which Mooser and Pearson have directed attention, but is also a result of the use of the term 'effective ionic charge'. Since these authors also use this expression without defining it, their timely article will not decrease the confusion on this point. As an example of the way in which effective ionic charge can be determined physically, they instance the dipole moment μ of a hydrogen halogenide molecule for which the nuclear separation is r_0. The effective ionic charge is then taken to be $Ze = \mu/r_0$, from which it is apparent that the definition of effective ionic charge which they intend may be "the value for the ionic charge deduced from a physical measurement on the basis of an over-simplified model or theory". The purpose of the present communication is to point out that the effective charge which can be deduced from certain physical measurements on crystals is clearly not the ionic charge, although it has been used as such[2].

I shall confine attention to crystals isomorphous with zinc blende. Methods of measuring the ionic charge which have been suggested may be classed as: (*a*) miscellaneous, (*b*) X-ray diffraction, and (*c*)

those depending for their interpretation on the theory of lattice dynamics. Under (*a*) we may instance studies of microcleavage[3], precise measurements of the wave-lengths of the $K\alpha_1$ and $K\alpha_2$ lines of sulphides[4], and paramagnetic resonance experiments[5] on zinc blende–manganese. None of these gives a quantitative result. Accurate X-ray measurements on zinc blende have been made by Jumpertz[6], who claims to have shown that the zinc atom carries a charge of $1{\cdot}29|e|$. This result can be disregarded since it was obtained by deriving individual atomic scattering factors $f(s)$ from the measured structure factors, and is therefore entirely dependent on the way in which the empirical curve for $f_{Zn}(s)$ is extrapolated to $s = 0$.

The ionic charge can in principle be determined unambiguously by X-ray diffraction only when a boundary can be drawn around the ion so as to pass through regions of the unit cell in which the electron density is small compared with the reciprocal of the volume enclosed. This will seldom, if ever, be the case: it can never be the case in a crystal in which there is appreciable covalent bonding. It may, of course, be possible to measure the charge in some arbitrarily specified volume, and to relate this to the ionic charge on a theoretical basis.

There remain the methods which we may class as 'lattice dynamical'. These include the comparison of measured and calculated cohesive energy, dielectric constant, elastic constants, piezoelectric modulus and infra-red absorption and reflectivity. The theory involves several approximations; in particular it is usually assumed either that the electronic polarizability of ionic crystals can be neglected, or that the electronic dipole moment of an atom is directly proportional to the Coulomb field[7]. On either assumption; (*a*) the elastic constants C_{11}, C_{12}, C_{44}; (*b*) the piezoelectric modulus e_{14}; (*c*) the difference $\varepsilon_s - \varepsilon_\infty$ between static and high-frequency dielectric constants: (*d*) the ratio ω_L/ω_T of the frequencies of longitudinal and transverse optic modes of vibration (which determines the width of the band of high reflectivity in the infra-red); (*e*) the occurrence of infra-red absorption at the frequency ω_T, all involve the ionic charge Ze. Either assumption, however, leads to unsatisfactory results when the frequencies of the normal modes of vibration are calculated for alkali halides, and results in considerably better agreement with experiment[8] are obtained for sodium iodide if it is assumed that the dipole moments also depend on the short-range exchange forces between the atoms, and conversely. Similar calculations have recently been made[9] for crystals of the zinc blende type, and show that the charge determining (*c*), (*d*) and (*e*) is an effective charge $Z'e$ which differs from the ionic charge by an amount which depends on the difference of the electronic polarizabilities of the two atoms concerned, and on the values of parameters describing the short-range interaction, as for the alkali halides. It is therefore possible to have $Z = 0$, but $Z' \neq 0$; that is, for a physically non-ionic crystal to exhibit properties which have hitherto been regarded as a test of ionicity. This unexpected conclusion is confirmed by the recent work of Tolpygo[10], whose earlier work in this field[11] has been overlooked in more recent theories[12] of the relation between Z and Z' in alkali halides. In general the piezoelectric modulus is found not to be directly proportional to Z, although it may be under certain conditions. Only the elastic constants C_{11} and C_{12} are found to be independent of the electronic polarizability and of the parameters which describe the short-range interaction between electronic dipole moments and atomic displacements. Detailed results will not be given here; but it seems worth mentioning that the values of the elastic constants of gallium arsenide and of similar III–V compounds, and a comparison of these values with those of germanium, indicate that Ga^0As^0 is a much better physical description than is Ga^+As^-, and that the values of Z' which have been reported[13] are not inconsistent with this conclusion.

Finally, we may note that, in at least one other branch of solid-state physics, ionic charges are deduced by gross over-simplification. It appears to be generally assumed that the spontaneous polarization of a ferroelectric is given by:

$$P = \sum_j Z_j e u_j$$

where u_j is the displacement of the j^{th} atom from its position in the unit cell of the phase of higher symmetry. It has, however, been shown[14] that the charges defined by this equation are apparent charges the relation of which to the ionic charges is quite complicated.

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Uranium Disilicide

A URANIUM–SILICON phase with a hexagonal *C*32-type structure, described by Zachariasen[1] as stoichiometric β-USi_2 and thought by Kaufmann, Cullity and Bitsianes[2] to be deficient in silicon, was shown by us[3] to be $USi_{1\cdot67}(U_3Si_5)$. At the same time attention was directed to the existence of another phase with the *C*32 (AlB_2)-type structure which was shown to be stoichiometric USi_2. This phase is isostructural with β-$ThSi_2$.

A further phase described as α-USi_2 by both Zachariasen and Kaufmann *et al.* has been found by us to occur at approximately 65 atomic per cent silicon. Arc-melted alloys containing 66·7 atomic per cent silicon consisted of this phase and USi_3, in the ratio of about 5 : 1 by weight. These results suggest that this phase is non-stoichiometric and has the formula $USi_{1\cdot88}$. This composition corresponds to one vacant silicon lattice-site in each pair of tetragonal structure cells.

Further experiment has shown that hexagonal USi_2, prepared by reaction of the elements in liquid bismuth, is stable only up to 450° C. Above this temperature USi_3 is obtained as the product of reaction in the liquid. When hexagonal USi_2 is heated alone above 450° C., mixtures of $USi_{1\cdot88}$ and USi_3 are obtained. The relative proportions of the two phases are the same as in the arc-melted alloy

Table 1

Phase	Composition in at. percentage silicon	Structure type and space group	Structure cell dimensions (Å.)
$USi_{1\cdot67}$	62·5	Hexagonal defect $C32$-type $C6/mmm$.	$a_o = 3\cdot843 \pm 0\cdot001$ $c_o = 4\cdot069 \pm 0\cdot001$
$USi_{1\cdot88}$	65	Tetragonal defect Ce-type $I\,4/amd$.	(i) heat treated below 650° C. $a_o = 3\cdot930 \pm 0\cdot001$ $c_o = 14\cdot06 \pm 0\cdot01$ (ii) arc-melted alloy $a_o = 3\cdot948 \pm 0\cdot002$ $c_o = 13\cdot67 \pm 0\cdot01$
USi_2	66·7	Hexagonal $C32$-type $C6/mmm$.	$a_o = 4\cdot028 \pm 0\cdot001$ $c_o = 3\cdot852 \pm 0\cdot001$

containing 66·7 atomic per cent silicon. No transformation comparable with that of β- to α-$ThSi_2$ [3,4] has been found as a result of heat-treating hexagonal USi_2.

Slight differences have been noted between the X-ray powder patterns of $USi_{1\cdot88}$ heat-treated above and below 650° C. This is considered to be a consequence of differences in ordering of the silicon atoms on the available lattice sites.

Crystallographic data for the three related uranium–silicon phases are summarized in Table 1.

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GEOCHEMISTRY

Measurement of the Thorium Content of Natural Materials by a γ-Ray Counting Method

Recent work by S. Raboy and C. C. Traul[1] on the background of sodium iodide scintillation counters suggested to us that it should be possible to measure the thorium content of a rock directly by counting the 2·62 MeV. γ-ray emitted in the decay of thorium C'' (^{208}Tl) to stable lead-208, since this γ-ray is more energetic than any in the uranium series. Through the generosity of the American Government a large sodium iodide crystal and suitable photomultiplier and the necessary electronic equipment were available to us to try out the method.

In an ordinary igneous rock the thorium content is usually about three times the uranium content. Fig. 1 shows the high-energy end of the γ-ray spectra of the thorium and uranium families, there being three times as much thorium as uranium. It will be seen that by using a wide-window single-channel analyser with its lower gate set at channel 25 on the spectrum, and its upper gate at channel 39, practically only the thorium C'' γ-ray will be counted. It was found experimentally that for a typical rock with a thorium/uranium ratio of three about 3 per cent of the net count in the chosen window was due to the uranium family. If the uranium content of the rock is known, the necessary correction to the observed net count can readily be made.

The sodium iodide crystal used was $4\frac{3}{8}$ in. in diameter and 1·8 in. deep, coupled to an E.M.I. 9530*B* fused silica photomultiplier with a cathode 4·4 in. in diameter. A special container was made so that the bottom and sides of the crystal could be surrounded by a $\frac{1}{2}$-in. layer of powdered material. This container held about 600 gm. of powdered rock.

It was found that there was a large thorium count in the background, apparently due to the thorium content of the granite walls of the building. By surrounding the crystal and photomultiplier with 4 in. of lead this was practically eliminated, but a trace was left, probably due to the small amount of thorium in the materials inside the shield.

The apparatus had to be standardized by using a sample of known thorium content. This was made by mixing pure thorium oxide containing 2 gm. of thorium with 1 kgm. of a sand of low thorium content. The oxide came from Joly's laboratory and is at least 50 years old. Since mesothorium-1 is the longest-lived daughter element in the thorium family with a half-life of 6·7 years, the oxide must be practically in radioactive equilibrium. It was found that a certain quantity of thoron escaped from this standard, consequently the standard sample holder was hermetically sealed. The count obtained per gm. of thorium is about 1,370 counts/min. 1 gm. thorium-232 produces $2\cdot16 \times 10^5$ disintegrations/min., but since only 34 per cent of these lead to the thorium C'' branch, the total theoretical 2·62-MeV. γ-ray emission should be $7\cdot35 \times 10^4$/min. Thus only about 2 per cent of this theoretical total is counted in the 2·62-MeV. peak. This result is in reasonable agreement with the efficiency as determined by K. J. Van Oostrum and A. C. Meijer[2] for a large sodium iodide crystal and E.M.I. photomultiplier.

The background is about 4·0 counts/min., and 600 gm. of a rock containing 10^{-5} gm. thorium/gm. gives a net count of 8·2/min., that is, about double the background count. The minimum quantity of thorium which can be measured is assumed to be that which will give a net count equal to four times its standard deviation. This gives a value of $0\cdot04 \times 10^{-5}$ gm. thorium/gm. of sample if 600 gm. of the specimen is counted for 20 hr.

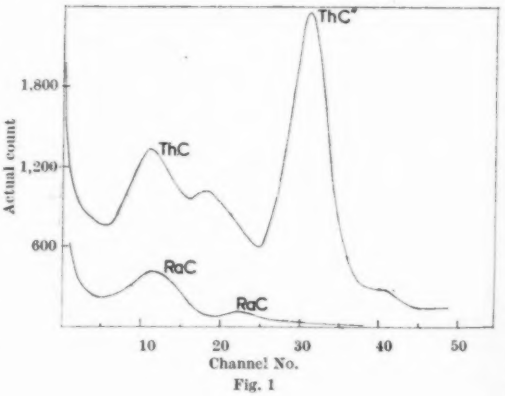

Fig. 1

Table 1

Material	Thorium 10^{-5} gm./gm.	
	γ-Ray method	Flow method
Average for six Deccan basalts	$0{\cdot}25 \times 10^{-5}$	$0{\cdot}46 \times 10^{-5}$
Basalt from Idaho Falls, Snake River, Oregon	$0{\cdot}19 \times 10^{-5}$	$0{\cdot}50 \times 10^{-5}$
Rapakivi Granite, Finland	$3{\cdot}03 \times 10^{-5}$	$6{\cdot}00 \times 10^{-5}$
Eclogite, Eppenreuth, Bavaria	$< 0{\cdot}03 \times 10^{-5}$	$0{\cdot}30 \times 10^{-5}$
Wicklow Granite, Ireland	$1{\cdot}15 \times 10^{-5}$	—
Globigerina Ooze, 2,493 fm., 48° 17′ N., 39° 49′ W.	$0{\cdot}32 \times 10^{-5}$	—
Globigerina Ooze, 720 fm. 38° 46′ W., 28° 31′ N.	$0{\cdot}41 \times 10^{-5}$	—

There are two chief objections to this method: first, a comparatively large sample is required; secondly, the requisite apparatus though entirely standard is moderately expensive. Unfortunately, most of the specimens the thorium content of which had already been measured by flow methods were too small to use. Some of the results we have obtained so far are given in Table 1.

The values given for the flow method were those obtained on the same specimens before 1930 and confirmed the modern view that such values were too high. The results for the Wicklow granite and for the Deccan basalts agrees very well with the modern estimate for the average thorium contents of such rocks.

We include the figures for the Globigerina oozes, since they seem to show that thorium is present in the oceanic deposits. The first specimen is situated west of the Atlantic Ridge, and the second is on the ridge and quite close to the Azores. It will be noticed that the shallower water specimen gives a considerably higher value These samples were dredged up by the cable ship *Faraday* in 1907. E. Picciotto and S. Wilgain[3] have given values varying from $0{\cdot}41 \times 10^{-5}$ gm. thorium/gm. to $0{\cdot}85 \times 10^{-5}$ gm. thorium/gm. for red clays from the Central Pacific cores of the Swedish Deep-sea Expedition 1947–48.

In conclusion, we may mention that P. M. Hurley[4] has also used a γ-ray spectrometer method for estimating the thorium content of rocks. He, however, worked at the lower-energy end of the spectrum, which means that the calculation of the results is much more complicated due to the greater contribution of the uranium series at these levels. It is of interest to note, however, that his result of $0{\cdot}28 \times 10^{-5}$ gm. thorium/gm. for a Deccan basalt agrees very closely with ours.

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METALLURGY

Resistivity Changes due to Formation of G. P. Zones (Clusters rich in Solute)

An anomalous increase in resistivity has been observed during formation of *G. P.* zones (clusters rich in solute) in several aluminium alloys[1,2]. Mott[3] suggested that this effect may be due to a critical scattering of the conduction electrons when the zone grows to dimensions of the wave-length of the conduction electrons. Matyas[4] showed on theoretical grounds that a maximum should occur for the resistivity change during zone formation.

The changes in resistance during zone formation in an aluminium–zinc alloy were discussed extensively in a recent paper by Panseri and Federighi[5]; the authors assumed that a zone of critical size is associated with the peak in resistance, and that the magnitude of the peak goes up with increasing number of zones.

The object of our research has been to test whether a zone of definite size is associated with the maximum in resistance which occurs during ageing. An alloy of aluminium–5·3 at. per cent zinc has been studied, using both resistance measurements and small-angle scattering of X-rays. For the resistance measurements, the resistance of a specimen 0·005 in. thick was measured at the temperature of liquid nitrogen. The specimen was quenched from 300° C. into water at 25° C. and aged at 0° C. or 30° C. Fig. 1 shows the variation in resistance with time. The resistance maximum occurs at 2 min. for ageing at

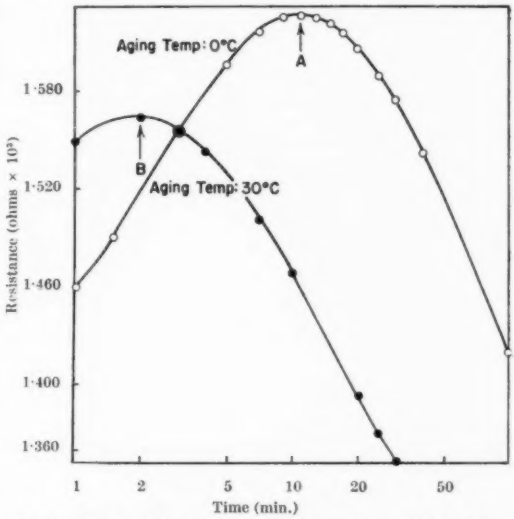

Fig. 1. Resistance in ohms versus log time for ageing at (*A*) 0° C. and (*B*) 30° C. after quenching from 300° C.; aluminium–5·3 at. per cent zinc

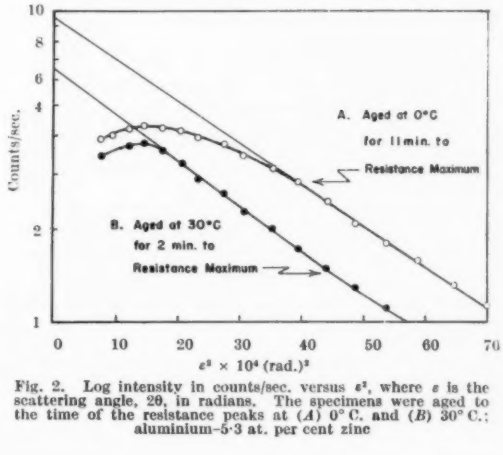

Fig. 2. Log intensity in counts/sec. versus ε^2, where ε is the scattering angle, 2θ, in radians. The specimens were aged to the time of the resistance peaks at (*A*) 0° C. and (*B*) 30° C.; aluminium–5·3 at. per cent zinc

30° C., and at 11 min. for ageing at 0° C. The magnitude of the peak at 0° C. is some 4 per cent higher than that of the peak at 30° C. Fig. 2 shows the small-angle scattering obtained from a specimen 0·005 in. thick of the same alloy and given treatments identical with the specimens used for resistance measurements. Ageing of the specimen was halted at the time of the peak in resistance by bringing it to −195° C. (liquid nitrogen), at which temperature the X-ray data were obtained. Balanced filters were used to obtain monochromatic copper $K\alpha$ radiation; step-scanning was employed due to the long exposures required. (The results are plotted as log I in counts/sec. versus ε^2, where ε is the scattering angle, 2θ, in radians.) The slope of the linear region to the right of the maximum gives the radius of gyration, R_g, of the spherical zone[6]. R_0, the zone radius, is given by $R_0 = (5/3)^{1/2} R_g$. R_0 is an average which emphasizes the largest zones, and since the scattering curves are not linear to the right of the peak (Fig. 2) there must be a range of sizes. The average radius can be expected to be about $\frac{1}{2} R_0$ for any reasonable distribution; this is 5 Å. for both the 0° C. and 30° C. resistance peaks. The maximum probable uncertainty in radius due to counting statistics and stability of X-ray output and counter was about 3 per cent.

The resistance maxima are then associated with zones of an average size of 10 Å. in diameter. Matyas's equation applied to the case investigated predicts about 7 Å. for the zone diameter. This is in reasonable agreement with what has been observed here experimentally: the results confirm the relation between the resistance maximum and zone size.

Re-ageing after reversion yields sizes closely the same as those quoted above, despite the fact that the ageing kinetics are different. These results will be part of a more detailed publication.

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Porous Nickel Oxide

In the course of an investigation of the oxidation properties of nickel strip used in the manufacture of electron-tube cathodes, a phenomenon was observed which may be of general interest.

Two samples of nickel in the form of strip 0·002 in. thick were oxidized by heating in air for 5 min. at 1,000° C. Dilute nitric acid was used to dissolve the metal and provide small pieces of coherent oxide film about 0·0001 in. thick. Under microscopic examination, the film from one sample was found to be apparently homogeneous and entire (Fig. 1*a*), while the other contained pores or holes about 1μ in diameter (Figs. 1*b*, *c*). The only distinguishing characteristic of the strip which produced this porous oxide film was that it contained about 0·02 per cent aluminium; the other strip contained about one-tenth of that amount.

a *b* *c*

Fig. 1. Electronmicrographs of nickel oxide films formed on: (*a*) nickel containing 0·002 per cent aluminium (× *c*. 390); (*b*) nickel containing 0·02 per cent aluminium (× *c*. 390); (*c*) nickel containing 0·02 per cent aluminium (× *c*. 1,880)

It is possible that small crystals of alumina are formed similar to those found by Allison and Samelson[1] when they oxidized nickel in wet hydrogen. These crystals may have fallen out of the nickel oxide layer when the nickel was dissolved, leaving the sharp-cornered holes in the oxide.

I am indebted to Dr. E. P. Bertin for the electronmicrographs.

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CHEMISTRY

Infra-Red Spectra of Water-soluble Carbonates

Infra-red spectra of some carbonates have been reported by Hunt, Wisherd and Bonham[1], Underwood, Toribara and Neuman[2] and by Miller and Wilkins[3]. Hunt *et al.* suggested that the position of the band near 11μ was a function of the atomic weight of the cation, and also pointed out that Miller and Wilkins's work substantiated this suggestion with the exception of lithium carbonate. We have evidence that this is not so. Further, Underwood *et al.* reported the 11μ band for sodium and potassium at different positions from that reported by Miller and his workers. Although they indicated their results were in general agreement with Miller and Wilkins, no attempt to point out or explain this discrepancy was made. Finally, the role of water in influencing both band positions and broadness appears to have been neglected in interpreting spectra of water-soluble inorganic compounds and this will be discussed here.

We find that for the soluble carbonates (sodium, potassium, rubidium and cæsium), this band appears at 11·4μ when they are in the anhydrous state and 11·6μ when they are hydrated. The band is therefore not a function of the atomic weight of the cation (at least for the soluble carbonates) but of the degree of hydration. As the degree of hydration is increased the band decreases in intensity and disappears when complete solution is obtained. No attempt was made to study which hydrate exists when the band shifts from 11·4 to 11·6μ, but it has been found that only slight traces of water are required, especially for the more soluble carbonates of rubidium and cæsium, to produce this shift. Decius[4] has related this position of the band for calcium carbonate to its crystal structure.

The band at 6·95μ for the anhydrous carbonates is shifted to 7·2 when complete solution is obtained. The intermediate states of hydration, however, give both bands, and, depending on the concentration of

water, one appears as a shoulder on the other, or they are broadened into one band appearing midway between the two. Thus the broadness and position of this band depend to a great extent on the water content, a fact apparently not recognized by many workers: although Miller and Wilkins, in their work, suggested that this possibility existed, they carried out no further investigation of it.

Attention should be directed to a band in the 9–10μ region for some of the carbonates. This band appears in strontium and lead carbonates in the work reported by Hunt *et al.* and also appears at 9·55μ in the spectra of sodium, potassium, rubidium and cæsium carbonate. According to Gatehouse *et al.*[5], this band appears only for carbonates having the aragonite structure.

These results were obtained in 'Nujol' and fluorocarbon mulls, potassium bromide pellets, powders dispersed on salt plates (for sodium carbonate only) and using physically sorbed water of synthetic zeolites as a solvent. This latter technique is useful for studying varying concentrations of water on carbonate spectra and was carried out in apparatus described elsewhere[6]. The problem of exchange in all these media was also studied and will be reported later.

We thank David Teloh for recording some of the spectra used in this work. One of us (R. P.) received a grant from the Petroleum Research Fund of the American Chemical Society.

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Electron Spin Resonance of Adenosine Triphosphate

IT has been suggested that in adenosine triphosphate (ATP) the phosphate chain and the adenine are linked by the pentose, so that the two terminal phosphates touch the two nitrogen atoms at positions 6 and 7 in the adenine ring[1]; and that, since triphosphate compounds might be electron acceptors and adenine is a good electron donor, the adenine group might donate an electron to the triphosphate group[2]. I. Isenberg and A. Szent-Györgyi[3] obtained an electron spin resonance signal from ATP (di-sodium salt) which they attributed to internal charge transfer between the adenine and the triphosphate. They also suggested that there was a possibility that the signal could be due to a transition metal impurity in the form of a transition metal – ATP complex. In view of the great importance of ATP in living processes, we have re-examined the electron spin resonance of this compound to decide between these possibilities.

The electron spin resonance spectrometer used in these experiments operates at 9·42 kMc./s. and has a conventional phase-sensitive detector system with a field modulation of 280 c./s. In these experiments the spectrometer sensitivity is 10^{13} ΔH spins, where ΔH is the line-width of the resonance signal of the material under investigation.

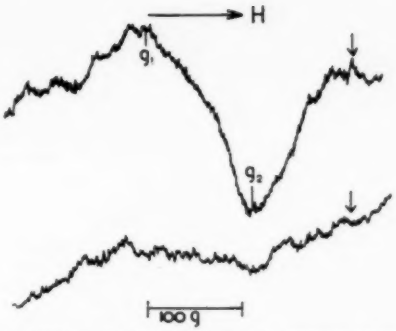

Fig. 1. First derivative of electron spin resonance absorption. Upper curve is commercial ATP, lower curve is purified ATP. The arrow indicates the free electron resonance

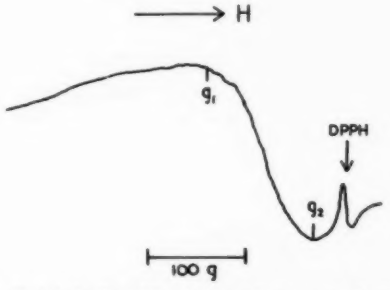

Fig. 2. First derivative of the electron spin resonance absorption of copper phthalocyanine

When commercial ATP (di-sodium salt) similar to that used by I. Isenberg and A. Szent-Györgyi was examined in the spectrometer, an anisotropic line of several hundred gauss line-width was obtained (Fig. 1). The full line shape could not be recorded as the largest field swept was 460 $\pm$ 20 gauss. Two arbitrary g values, $g_1 = 2{\cdot}128 \pm 0{\cdot}010$ and $g_2 = 2{\cdot}064 \pm 0{\cdot}004$, where g_2 is approximately equal to $g_\perp$ and g_1 is related to $g_\parallel$, were measured. These values are significantly higher than the free electron value of 2·0023. The position and shape of the signal indicated the presence of copper bound in some way to the nitrogen atoms of the adenine group. The ATP was therefore analysed for copper, which was estimated colorimetrically at 435 mμ with diethyldithiocarbamate[3], and the amount found was 75·2 $\pm$ 4·0 μgm. copper/gm. ATP.

Copper phthalocyanine was prepared to test whether copper bonded to nitrogen was responsible for the electron spin resonance signal observed in ATP. The copper phthalocyanine gave a spectrum of the same anisotropy as the ATP spectrum. Two arbitrary g values were measured as before, $g_1 = 2{\cdot}087 \pm 0{\cdot}004$ and $g_2 = 2{\cdot}025 \pm 0{\cdot}001$. The differences between the values of g_1 and g_2 for ATP and copper phthalocyanine are the same, which suggests that the resonance signal observed in ATP is due to copper, the differences in the absolute values of g_1 and g_2 in the two cases being due to slight differences in the chemical bonding of the copper.

To confirm this suggestion, a known weight of copper phthalocyanine, diluted with aluminium oxide,

was placed in a quartz tube, so that the length of the sample was the same length as the ATP sample, and a spectrum recorded. The amount of copper present in the ATP was calculated from the relative signal sizes, assuming that the shapes of both signals were the same. The value obtained was 83·5 ± 13·5 μgm. copper/gm. ATP, which agrees within experimental error with the figure of 75·2 ± 4·0 μgm. copper/gm. ATP obtained by the diethyldithiocarbamate method. A second sample of commercial ATP gave the values 71·5 ± 4·0 μgm. copper/gm. ATP by the diethyldithiocarbamate method and 70·4 ± 10·1 μgm. copper/gm. ATP from electron spin resonance measurements. A sample of ATP purified by the method of Dubois, Albaum and Potter[4] was found to contain 17·8 ± 4·0 μgm. copper/gm. ATP by the diethyldithiocarbamate method, which agrees with that obtained by electron spin resonance measurements (17·8 ± 2·9 μgm. copper/gm. ATP).

These results show that the electron spin resonance signal observed in ATP is most probably due to copper impurity present as a metal-ATP complex. Other work indicates that the complex is very stable (Russell, D. B., unpublished work) and that ATP forms stable complexes with iron (Russell, D. B., unpublished work) and manganese[5]. If charge transfer does occur between the adenine and the triphosphate, there is insufficient unpairing of electron spin for this to be observed on the electron spin resonance spectrometer used.

We thank Dr. P. J. Warren, of the Department of Chemical Pathology of this hospital, who estimated the copper content of the ATP samples by the diethyldithiocarbamate method.

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Co-ordination of the Thiocyanate Group in Inorganic Compounds

There has been a rapid growth of interest in the study of the thiocyanate ion in inorganic compounds.

Previously we have investigated the vibrational behaviour of the thiocyanate group in several inorganic compounds[1]. Similar investigations have been carried out independently and with different compounds by Chamberlain and Bailar[2], Kinell and Strandberg[3] and Mitchell and Williams[4]. However, the last two authors have limited their work to the CN-stretching vibration.

There are three ways in which the di-functional thiocyanate ion can be attached to a metal cation, namely, *M*–SCN, *M*–SCN–*M* and *M*–NCS. They can be differentiated by the frequencies assigned to the fundamental stretching modes of the triatomic group, approximating to CN- and CS-stretching. In fact, we find that the CS-stretching frequency is more diagnostic, particularly in the absence of *M*–SCN–*M* 'bridges'. Moreover, the observation of the 5μ region, where the CN bands occur, is often particularly difficult when working with solid samples.

Table 1. Fundamental Stretching-Frequencies (cm.$^{-1}$) of the SCN Group in Some Thiocyanate Complexes*

Compound	ν_3 (SCN)	ν_1 (SCN)	Structure	Ref.
$Hg(SCN)_2$	2,118	716	—	*a*
$K_2Hg(SCN)_4$	2,115	716	Hg–S	*b, k*
$K_2Co(NCS)_4.4H_2O$	2,074 bd	822, 818	Co–N	*b*
$K_2Pt(SCN)_4$	2,089, 2,120	697 700 sh, 690 sh	Pt–S	*b*
$K_2Pd(SCN)_4$	2,118, 2,086 bd	703 707 sh, 696 sh	—	
cis-$Pt(NH_3)_2(SCN)_2$	2,116	703	Pt–S	*c*
trans-$Pd(NH_3)_2(SCN)_2$	2,116, 2,100	701	—	
$Pt(PEt_3)_2(NCS)_2$	2,113	855	—	
$Pt(PPr^i{}_3)_2(NCS)_2$	2,106	854	—	
$Pd(PEt_3)_2(NCS)_2$	2,089	846, 842 sh	—	
$Pd(PPr^i{}_3)_2(NCS)_2$	2,094	852 sh, 844	—	
$Zn(N_2H_4)_2(NCS)_2$	2,060 bd	790	Zn–N	*d*
$Zn\ py_2(NCS)_2$	2,096, 2,075	847	—	
$Mn(N_2H_4)_2(NCS)_2$	2,065	794	Mn–N	*d*
$Ni[SC(NH_2)_2]_2(NCS)_2$	2,080	810	Ni—N	*e*
$Ni[CH_3S(CH_2)SCH_3](NCS)_2$	2,085	805	—	*f*
$Ni(PR_3)_2(NCS)_2$	2,095	860	—	*j*
$K_2Pt(SCN)_4$	2,125, 2,115	694	Pt–S	*b, l*
$Cu\ py_4(NCS)_2$	2,094	824	—	
$Co\ py_2(NCS)_2$	2,066	800	Co–N	*g*
$Ni(NH_3)_2(NCS)_2$	2,102	778	Ni–N	*h*
$Ni\ py_4(NCS)_2$	2,080	798	Ni–N	*g*
$(NH_4)[Cr(NH_3)_2(NCS)_4]$	2,110, 2,042	828	Cr–N	*i*

* Spectra were observed in 'Nujol' mulls and measured with sodium chloride and calcium fluoride optics on Perkin–Elmer spectrometers (21 and 112 respectively).
(*a*) The X-ray structure determination of $Hg(SCN)_2.KSCN$ indicates Hg–S bonds and absence of a bridge structure; for ref. see under (*b*).
(*b*) Zhdanov, G. S., and Zvonkova, Z. V., *Doklady Mezhdunarod. Kongr. Teoret. i Priklad. Khim.*, XIII Kongr. Stockholm (1953), p. 175.
(*c*) See ref. 11.
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(*f*) The 805 cm.$^{-1}$ band reported by Sweeny, D. M., *et al.* (*J. Amer. Chem. Soc.*, **77**, 6521; 1955) is assigned here to the ν_3 vibration.
(*g*) Porai-Koshits, M. A., and Antsishkina, A. S., *Kristallografiya*, **3**, 686 (1958).
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(*i*) Saito, Y., Takeuchi, Y., and Pepinsky, R., *Z. Krist.*, **106**, 476 (1955).
(*j*) R = Et, Pr^n, Pr^i, *cyclo*hexyl.
(*k*) These results are in good agreement with those reported by Chamberlain and Bailar (ref. 2).
(*l*) Frequencies reported by Chamberlain and Bailar (ref. 2).

Our recent results are summarized in Table 1, together with those for other compounds examined by us[1].

An analysis of the CS-stretching frequencies ν_1(SCN) in compounds of known structure clearly indicates the infra-red criterion for the characterization of the metal-thiocyanate bond. The symmetrical band ν_1 occurs between 690 and 720 cm.$^{-1}$ for the $M \leftarrow$SCN compounds, and is in the range 780–860 cm.$^{-1}$ for the $M \leftarrow$ NCS compounds. An extension of this criterion to compounds of unknown structure listed in Table 1 leads to the following conclusions, which may be of general interest for class (*b*) metals.

The CS-stretching frequencies found in $[Pd(SCN)_4]^{2-}$ and $[Pt(SCN)_4]^{2-}$ are in agreement with *M*–S bonds, whereas in the complexes with the phosphines the metal must be bound to the nitrogen atom. It is worth noting that we also verified that sulphur binding in $[Pd(SCN)_4]^{2-}$ and $[Pt(SCN)_4]^{2-}$ places SCN^- in the same position as Cl^- in the spectrochemical series. The shift of the ultra-violet absorption bands towards higher frequencies (as compared with the frequencies of the chloride derivatives) is

fairly clear (1,500 cm.$^{-1}$) in the case of $Pd(PPr^i_3)_2(NCS)_2$, but is indistinguishable in $Pd(PEt_3)_2(NCS)_2$.

The change from *M*–S to *M*–N bonds when two SCN groups of $[M(SCN)_4]^{2-}$ are replaced by two phosphine molecules is not observed when the same SCN groups are substituted by two ammonia molecules giving $M(NH_3)_2(SCN)_2$. These results indicate that in complexes where SCN is not the sole ligand, the type of co-ordination of the thiocyanate group may depend on the nature of the second ligand partner. There must be a critical point where, with a particular ligand, change in the co-ordination can occur. It should then be possible, in the case of complexes with general formula $M(CNS)_mX_n$, to prepare two structural isomers of the same compound, depending on the correct choice of the *X* ligand or alternatively by changing the ratio $m:n$. No two such isomers with any particular metal has yet been reported. It has been suggested that the $[Ag(SCN)_2]^-$ ion in solution is an equilibrium mixture having Ag–N as well as Ag–S bonds[5]. However, further evidence to support this point seems necessary.

Previous workers have pointed out that a given metal ion becomes attached to one end of the SCN group rather than the other[6]. The binding is generally through sulphur in those cases where the stability sequence among the halides is $I^->Br^->Cl^->F^-$ (ref. 7) and the change from *M*–SCN to *M*–NCS bonding coincides approximately in the Periodic Table with the change in relative stability from the above sequence to $F^->Cl^->Br^->I^-$ (ref. 4). Some authors have pointed out that this is due to a change in the relative importance of covalent and ionic bonding in the compounds[4,7], whereas others have stressed the importance of the overlap between particular sets of orbitals in explaining the above stability series[6]. We believe that the π-orbitals of the thiocyanate group should not be neglected as a factor in the stabilization of the *M*–S bond.

There are two sets of antibonding π-orbitals in the thiocyanate ion and both are localized on the sulphur atom; use of the empty *d*-orbitals of sulphur can confer further acceptor properties on the sulphur end of the group. Interaction of d_π electrons on the metal atom with these empty orbitals will result in additional stability of the *M*–S bond. Whether or not this factor of additional stability will be of importance will depend on the availability of the d_ε electrons of the metal and on their relative energy as compared with that of the antibonding π-orbitals of the thiocyanate.

The change from *M*–S to *M*–N bonds for the complexes of platinum in Table 1 can be explained by considering that strong π-electron acceptors, as tri-substituted phosphines are believed to be, can make the d_ε electrons less available for binding with the π-orbitals of SCN, that is, for a bond on the sulphur end.

In the cases mentioned the effect of the phosphine molecules is to change the co-ordination from *M*–S to *M*–N. However, there are also compounds with the same ligands where the thiocyanate group still retains a distinct bridge configuration. In such cases one finds that the *M*–S bond is weakened, whereas the *M*–N bond apparently is not affected. A Pt–S bond-length (2·44 Å.) greater than the sum of the atomic radii (2·36 Å.) has been found by Owston and Rowe in $[Pt_2(SCN)_2Cl_2(PPr^n_3)_2]$, and this result has been explained with somewhat similar arguments to those adopted above[9]. Also, in the case of $AgSCN.PPr^n_3$, the Ag–S bond-lengths are considerably greater (2·75 Å.) than expected (2·43 Å. in AgSCN)[10]. These results can be compared with the 'normal' bond-lengths of 2·30 and 2·27 Å. reported in *cis*- and *trans*- $Pt(NH_3)_2(SCN)_2$ (ref. 11).

A broad generalization based on the few cases discussed here is obviously not possible; more experimental material is required in order to appreciate the factors affecting the change from *M*–SCN to *M*–NCS bonding. Nevertheless, our observations suggest that π-bonding may be of importance in balancing the equilibrium $M\text{–NCS} \rightleftarrows M\text{–SCN}$ far to the right for cations such as Pd(II) and Pt(II). If our interpretation is correct, the same change in co-ordination of the thiocyanate as observed for the complexes with the phosphines may be predicted to occur by different, strong π-electron acceptor ligands.

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Surface Symmetry and Cell Dimensions of Layer Lattice Silicates

In a previous communication[1] it was pointed out that the ditrigonal surface symmetry observed for a number of layer lattice silicates probably plays an important part in determining polymorphism among the micas. This idea has now been extended considerably by theoretical and experimental studies to be reported elsewhere. It appears to me that the ideal layer lattice structures must have ditrigonal, rather than hexagonal, surface symmetry. That is, the tetrahedral groups apparently can rotate quite readily about axes normal to the sheets to allow the tetrahedral sheets to contract to the dimensions of the octahedral sheets.

These concepts have several important implications. For example: (1) it is shown easily that the average tetrahedral rotation is given by $\alpha = \cos^{-1}(b_{obs.}/b_{tetr.})$, where $b_{obs.}$ is observed *b*-axis, and $b_{tetr.}$ is *b*-dimension which the given tetrahedral layer would assume, if unconstrained. This gives reasonable agreement to $\alpha_{obs.}$ for those structures for which the latter is known. Values of $\alpha_{calc.}$ for a large number of layer lattice silicate structures show that almost all these minerals have more or less ditrigonal surface networks, for example, α for micas = 6–23°.

(2) The substitution of aluminium for silicon in the tetrahedral layer will not increase $b_{obs.}$ but merely increase α, that is, change the surface configuration. Previous unit cell formulæ giving $b_{obs.}$ in terms of composition[2] are therefore wrong in this respect. Multiple regression analyses, of *b* against composition,

XUM

computed separately for experimental data for kaolins, micas and chlorites have each yielded non-significant regression coefficients for tetrahedral Al^{iv}, confirming the theoretical predictions.

(3) In the case of the micas, the interlayer cations play a major part in determining the unit-cell dimensions, a direct contradiction of current concepts. In general the 'Si'–O tetrahedra rotate until the nearer six oxygens (of the twelve) are 'in contact' with the interlayer cation, as I[3] have shown in the particular case of $2M_1$ muscovite. A large interlayer cation therefore contributes significantly to the value of $b_{obs.}$; interlayer K^+ has a greater effect than octahedral Fe^{++} for micas. This physical concept has been confirmed by the multiple regression analyses mentioned. A single relation obtained from the mica data, giving b in terms of composition, fits both normal and brittle micas adequately, and both dioctahedral and trioctahedral types. For example, the unusual brittle mica xanthophyllite has $b_{obs.} = 9{\cdot}01$ and $b_{calc.} = 9{\cdot}016$, whereas earlier formulæ break down altogether[2].

(4) It is obvious that the relative rigidity of the Si–O tetrahedral group takes control of the b-axis dimensions of those layer minerals for which the octahedral layer would be significantly larger than the tetrahedral layer. It is for this reason that $b_{obs.} = 9{\cdot}15$ for talc, rather than $\approx 9{\cdot}3$; and the same factor is possibly an important one in distinguishing the chrysotile from the antigorite structures.

Necessary experimental results to support these ideas are now being submitted for publication. Some of these data have been kindly supplied by Dr. K. Norrish of this Division, with whom I have had very profitable discussions of this work.

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R_M and Solvent Composition in Paper Chromatography

When information concerning the structure of substances is required from paper chromatographic data, R_M values are used rather than R_F as they are more directly related to the different groups of which the molecules of these substances are composed[1]. In cases when one phase forming the chromatographic system is composed of two solvents practically immiscible with the other liquid phase, at any proportion of the component solvents (for example, hexane + chloroform/water; hexane/water + dimethylsulphoxide) and assuming that chromatography is a true counter-current extraction process, following the plate theory, a simple equation can be derived relating the R_M value to the composition of the two-component phase.

In the systems mentioned, under ideal conditions, the partition coefficient of a substance between the mixed two-component phase (1 + 2) and the other phase is related to the volume fractions of the component solvents (u_1 and u_2) and to the partition ratios of the substance between the pure component solvents and the other phase (K_1 and K_2) by Kemula–Buchowski's equation[2]:

$$\log K = u_1 \log K_1 + u_2 \log K_2 \qquad (1)$$

Taking into account that $u_1 + u_2 = 1$ we can write:

$$\log K + \log r =$$

$$u_1 \log K_1 + u_2 \log K_2 + u_1 \log r + u_2 \log r$$

or

$$\log Kr = u_1 \log K_1 r + u_2 \log K_2 r \qquad (2)$$

(r is volume coefficient or 'paper constant' is ratio of cross-sectional areas of non-polar and polar phase.)

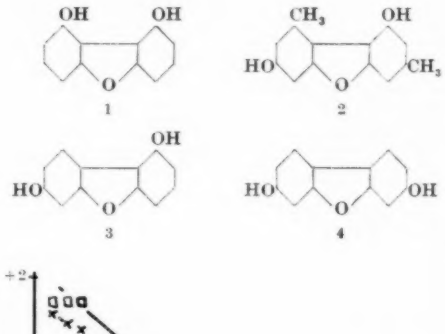
As K_1, K_2 and K are usually defined as ratios of concentrations of substance in the non-polar and polar phase, at equilibrium, then $R_M = -\log Kr$ in conventional paper chromatography and $R_M = \log Kr$ in reversed phase chromatography[1] so that in both cases:

$$R_M = u_1 R_{M1} + u_2 R_{M2} \qquad (3)$$

where R_M, R_{M1}, R_{M2} denote the R_M values of the substance in the systems: mixed solvent (1 + 2)/other phase; pure solvent 1/other phase; pure solvent 2/other phase.

If the volume coefficient r is constant and independent of the composition of the mixed solvent (1 + 2), the R_M value of a substance should thus be a linear function of the volume composition of the mixed solvent (this will also apply if r is an exponential function of volume composition, that is, $\log r = ku_1$).

In order to give an experimental illustration of this simple rule, in Fig. 1 some results from other work[3] are given, represented in the form of R_M versus volume composition plots. Experimental details are to be found in ref. 3.



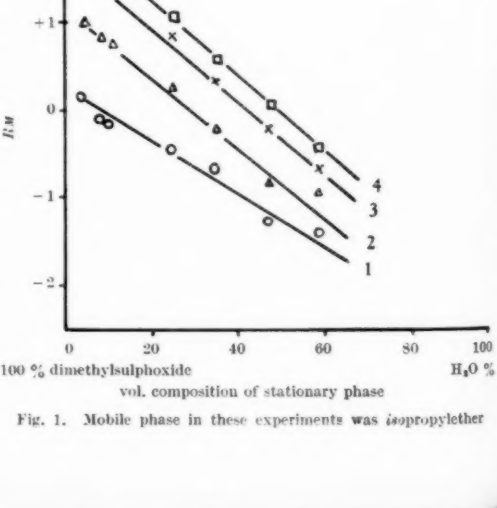


Fig. 1. Mobile phase in these experiments was *iso*propylether

It can be seen that for certain solvent systems results may be obtained in accordance with equation 3. Similar relationships can also be expected in cases when the paper is impregnated with a non-polar liquid (for example, paraffin oil) and developed with mixtures of water and alcohol, etc.

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BIOCHEMISTRY

Separation Methods based on Molecular Sieving and Ion Exclusion

CROSS-LINKED dextran ('Sephadex') has been shown to possess properties of a molecular sieve[1]. The size limits for effective molecular sieving depend on the degree of cross-linking. Molecules or ions capable of entering the gel phase may be subject to specific interaction with the matrix substance. Thus, for example, when certain aromatic compounds are passed through 'Sephadex' columns, a more extensive retardation is found than if the gel acted as a molecular sieve only.

It has now been found that, due to the superimposed sieving and sorption effects (the latter due to aromaticity and charge), polypeptides such as oxytocin and vasopressin are not easily separated from salts, amino-acids and many other substances of low molecular weight (with K_d about 1). It was thought that the separation factors in such cases would be increased if the swelling of the gel grains were more restricted. Following up this idea, we tried solvent systems that contained various proportions of acetic acid, pyridine and water. The swelling of the gel in such solvents is less extensive than in water, and, as expected, some compounds could be effectively excluded while others were distributed to different extents between the gel phase and the free liquid. The distribution is highly dependent on the concentration of acetic acid, which, when large effects are desired, should exceed 40 per cent by volume. In our experiments 15–30 per cent of pyridine was also included both to limit the swelling and avoid too high an acidity of the medium, and also to make effective desalting of peptides possible.

Fig. 1 shows two superimposed diagrams of similar filtration experiments made under the following conditions. Seven grams of low molecular weight substances from posterior lobes of hog pituitaries, prepared by gel filtration as described in an earlier publication[2], were dissolved in 50 ml. of a solvent mixture consisting of 60 parts of acetic acid, 15 parts of pyridine and 25 parts of water (by volume). The solution was filtered through a 'Sephadex' *G* 50 column, 65 cm. × 4·5 cm., packed and pre-washed in the same solvent[4]. The rate of flow was about 25 ml. per hr. Ninhydrin reaction[3] was determined in 0·05-ml. aliquots, from fractions of 12–13 ml. Similar results were obtained with 'Sephadex' *G* 25. It was found by other methods that the substances in fractions 1, 2 and 3 consisted mainly of peptides ; for example, vasopressin was found in peak 2. Fraction 5–7 consisted of amino-acids and other ninhydrin-reactive compounds.

Ion exclusion on xerogels is another principle for fractionation of electrolytes[4]. Using this principle, we have separated peptides on ion exchangers derived from cross-linked dextran (kindly put at our disposal by Dr. P. Flodin, Pharmacia, Uppsala).

As a principle for separation, ion exclusion, with the fixed ionizable groups completely titrated, is

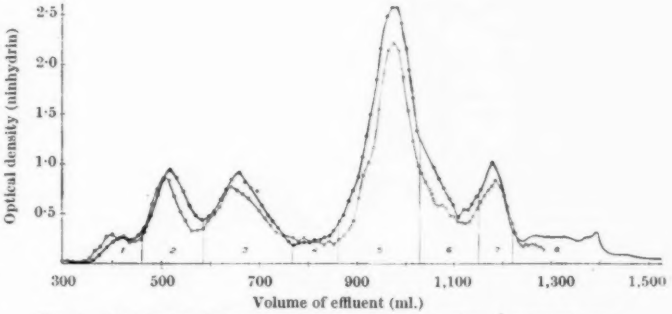

Fig. 1. Distribution diagrams of two similar gel filtration experiments on a 'Sephadex' *G* 50 column. Solvent system: 60 parts of acetic acid, 15 parts of pyridine and 25 parts of water

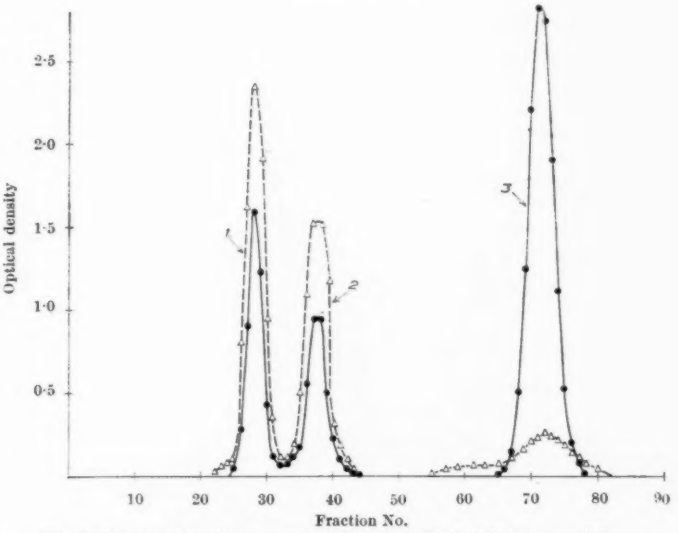

Fig. 2. Ion-exclusion diagrams on a DEAE-'Sephadex' *A* 25 column. Folin–Lowry reaction, –Δ–Δ–Δ–, and ninhydrin, –●–●–●–. Vasopressin is found in peak 1 and oxytocin in peak 2

probably in some respects superior to ion exchange. The contact with the ion exchanger is less active, since intimate interaction with strong adsorption centres is avoided. The capacity appears to be larger and the reproducibility better than is generally the case for ion-exchange chromatography, in which eluants of constant composition are employed. However, the resolution is possibly inferior to what may be achieved in ion-exchange chromatography. The ion or molecular sieving confers a property on the charged gel that converts ion exclusion into something more than merely an inversion of exchange. The sorption or exclusion of ions and molecules is chiefly based on the interplay of restricted diffusion and electrostatic repulsion.

Fig. 2 illustrates ion exclusion of neurohypophyseal peptides that originally were bound to proteins[2]. The experiment was carried out on a diethylaminoethyl-ether derivative of cross-linked dextran (DEAE-'Sephadex' *A* 25, 2·4 m.equiv./N/gm. dry weight : water regain, 5·5 ; particle size, 200–400 mesh). 250 mgm. peptide material were dissolved in 5 ml. of 0·1 molar pyridinium acetate and introduced into a 95 cm. × 3·5 cm. column bed. The chromatogram was developed in 0·1 molar pyridinium acetate at a speed of 30 ml. per hr. After 90 ml. eluent had passed into the column, 7·5 ml. fractions were collected. Aliquots of 0·1 ml. were analysed with ninhydrin and 0·2 ml. aliquots by the Folin–Lowry method[5]. The reproducibility is excellent. Peak 1 contains lysine vasopressin (150–200 international rat blood-pressure units per mgm.), and peak 2 chiefly consists of oxytocin (300–400 international avian blood-pressure units per mgm.). Negatively charged ion species are of course adsorbed and may be eluted at a later stage either with the same eluant (if the sorption forces are weak enough) or by displacement.

The outcome of a number of preliminary experiments made under other conditions and with other substances indicates that the methods described here ought to find applications in many fields.

Further details of this work will be published later.

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Simplified Procedure for removing Non-specific Staining Components from Fluorescein-labelled Conjugates

Coons and Kaplan[1] in their description of the fluorescent antibody technique using antisera coupled with fluorescein isocyanate found that the non-specific staining which occurred when such conjugates were applied to tissue sections could be minimized by dialysing the conjugates against 0·01 *M* phosphate buffer solution *p*H 7·0 for several days, followed by treatment with liver powder.

Marshall *et al.*[2] showed that fluorescein isothiocyanate was superior to the isocyanate in this technique, and in their description of the simpler conjugating procedure which is possible using this compound they also dialysed against buffer *p*H 7·0 to remove non-specific staining components from the solution.

It has been found that non-specific staining material in conjugates prepared from fluorescein isothiocyanate can all be removed by serial treatment with liver suspension alone, thus eliminating the long dialysis procedure.

In these experiments the following systems were employed : (1) human group '*A*' stomach sections treated with rabbit anti-'*A*' serum[3] followed by goat anti-rabbit γ-globulin conjugate ; (2) calf thyroid sections treated with lupus erythematosus serum containing antinuclear factor[4] followed by rabbit anti-human γ-globulin conjugate. In addition to these 'sandwich' tests the conjugates were also applied direct to the sections, all of which were cut in a cryostat.

Conjugates of crude globulin preparations were prepared according to the method described by Marshall *et al.*[2], and after overnight mixing in the cold aliquots of the reaction mixture were treated : (1) by dialysis against buffer *p*H 7·0 as described by Coons and Kaplan[1], followed by a single absorption with liver suspension ; (2) by serial treatment with liver suspension. The anti-rabbit conjugate was treated with human liver and the anti-human conjugate with rabbit liver. The liver suspensions were prepared by suspending the dried powder, made by the method of Coons and Kaplan, in *p*H 7·0 buffered saline, centrifuging, and the excess fluid decanted. The use of such a moist suspension eliminates the considerable loss of conjugate which occurs when dry powder is used. Equal volumes of the packed suspensions were added to aliquots of the conjugates and the mixtures slowly rotated for 10 min. at 4° C. The supernate was removed after centrifugation and for serial absorption was treated with a further volume of suspension ; after mixing for 10 min. and centrifuging, the supernate was finally treated with a third volume of suspension.

The results obtained by treating sections with the various conjugate preparations are summarized in Table 1.

Table 1. Effect on Non-specific Staining and Agglutinin Titre of Dialysis and/or Treatment with Liver

Treatment	Non-specific staining	Specific staining	Agglutinin titre of anti-human γ-globulin conjugate
Dialysis + 1 liver treatment	Very slight	Bright	1,280
Undialysed 1 liver treatment	Moderate	Bright	1,280
Undialysed 2 liver treatments	Very slight	Bright	1,280
Undialysed 3 liver treatments	Minimal	Moderate	640

It will be seen that two treatments with liver suspension resulted in conjugates which gave satisfactory specific staining and no more non-specific staining than the conventional procedure. Conjugates treated three times with liver showed least non-specific staining but detectable reduction in the brightness of the specific staining. This latter observation was confirmed by titrating the various preparations of the anti-human γ-globulin conjugate for their agglutinating activity against human *Rh*+ erythrocytes sensitized with incomplete anti-*D* antibody. The titres obtained are shown in Table 1.

In the orthodox procedure one of the effects of dialysis is to reduce the *p*H of the mixture from 9·0 to 7·0. It therefore seemed possible that the failure of one liver treatment to bring about an adequate reduction in non-specific staining might be attributable to the higher *p*H of the undialysed solution.

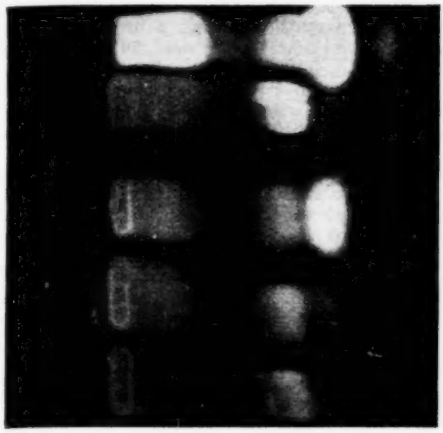

Fig. 1. Electrophoresis of fluorescent conjugate solutions, carried out on cellulose acetate membranes in borate-barbitone buffer, *p*H 8·6 at about 15 V./cm. *A*, albumen position; *γ*, *γ*-globulin position; *U*, untreated; *D-L*, dialysed and treated once with liver suspension; *L*-1, *L*-2, *L*-3, one, two and three liver treatments respectively of the undialysed conjugate

That this is not so was shown by adjusting the reaction of an undialysed conjugate to *p*H 7·0 by the addition of phosphoric acid. After one treatment with liver suspension this preparation gave as much non-specific staining as the same conjugate treated with liver at *p*H 9·0.

Electrophoresis of the conjugate preparations was carried out on cellulose acetate membrane at about 15 V./cm. in borate-barbitone buffer, *p*H 8·6 for 1 hr. The strips were photographed in ultra-violet light and the results are shown in Fig. 1. The untreated conjugate gave a serum protein pattern on which two bright zones were superimposed, one just ahead of the albumin, the other in the β–α_2-globulin region. It is evident that dialysis followed by a single liver treatment, and serial liver treatment alone, have similar effects on the electrophoretic patterns obtained, the brightest two zones being considerably reduced by both procedures. It is of interest that the γ-globulin components of the conjugates constitute a very small proportion of the fluorescent material present, although when these strips were stained with protein dyes the γ-globulin stained strongly and was a major component. It therefore appears that the fluorescein reagent has a greater affinity for proteins other than γ-globulin.

On the basis of these results serial treatment with appropriate organ suspension alone appears to give satisfactory conjugate preparations for staining tissue sections.

The electrophoresis was carried out by Dr. R. Consden, and it is a pleasure to acknowledge his help and interest in this work.

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Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue

It has been established that, whereas the usual diphosphopyridine nucleotide (DPN)-linked glyceraldehyde-phosphate dehydrogenase is found generally distributed in higher plant tissues, a separate enzyme, specifically requiring triphosphopyridine nucleotide (TPN) as coenzyme, is restricted to leaves[1–4]. The distinctive association of the TPN-glyceraldehyde phosphate dehydrogenase with the photosynthetic apparatus and the specificity for TPN shown in some types of photosynthetic phosphorylation have encouraged the view that the reductive step in hexose synthesis in leaves is mediated by this enzyme.

An active hexose synthesis from acetate which also involves a reversal of the glycolytic sequence occurs in the colourless endosperm tissue of some germinating fatty seedlings. It was therefore of interest to find that in this non-photosynthetic tissue both types of glyceraldehyde phosphate dehydrogenase are present.

Extracts were prepared in sucrose phosphate mixtures from endosperm tissue of castor beans which had been grown in the dark for 4–5 days (see ref. 5). Enzyme activity of the supernatant solution after centrifuging at 18,000*g* was measured by the procedure outlined by Gibbs[6] in which reduction of the coenzyme is followed in the presence of fructose-1,6-diphosphate (to provide substrate through the action of aldolase), arsenate and fluoride. The results in Table 1 show that both TPN and DPN are reduced and that the rate of reduction increases with enzyme concentration. The maximum rates achieved with DPN are considerably higher than those with TPN. The results in Table 2 show the effect of increasing substrate concentration on enzyme activity. It is seen that the TPN enzyme is saturated at lower substrate-levels than the DPN enzyme.

Table 1. Effect of Increasing the Amount of Enzyme Extract on the Initial Rates of DPN and TPN Reduction

The reaction mixtures contained, in micromoles: *tris* buffer (*p*H 8·5) 100; sodium arsenate, 51; sodium fluoride, 60; enzyme extract and (DPN, 0·15 plus fructose-1,6-diphosphate, 2·6) or (TPN, 0·18 plus fructose-1,6-diphosphate, 0·3). Total volume, 3·0 ml.

Enzyme added (ml.)	TPN reduction (Δ O.D.$_{340}$/min.)	DPN reduction (Δ O.D.$_{340}$/min.)
0·025	0·004	0·012
0·050	0·0145	—
0·080	0·025	—
0·10	0·030	0·048
0·20	—	0·081
0·30	—	0·089

Table 2. Effect of Increasing Amounts of Substrate on DPN and TPN Reduction

Reaction mixtures as in Table 1, with 0·1 ml. enzyme solution and fructose-1,6-diphosphate as indicated

Fructose-1,6-diphosphate (μmoles)	TPN reduction (Δ O.D.$_{340}$/min.)	DPN reduction (Δ O.D.$_{340}$/min.)
0·0	—	0·003
0·056	0·016	—
0·112	0·020	—
0·168	0·0205	—
0·187	—	0·021
0·224	0·0225	—
0·370	—	0·031
1·30	—	0·046
2·60	—	0·049

The possibility that TPN reduction was due to glucose-6-phosphate dehydrogenase rather than glyceraldehyde-phosphate dehydrogenase was eliminated by the following observations:

(*a*) The rate of reduction of TPN was much lower when glucose-6-phosphate was used instead of fructose-1,6-diphosphate. (*b*) The reduction was

completely inhibited by 0·001*M* iodoacetate. (c) Reduction of TPN was observed when glyceraldehyde-3-phosphate was used directly as substrate.

In crude extracts TPN reduction does not require the addition of arsenate or phosphate (cf. ref. 6). In experiments with a partially purified preparation, which contains relatively greater amounts of the TPN enzyme, arsenate stimulations are observed. Nevertheless, a considerable fraction of the TPN reduction appears to be catalysed by a third (non-reversible) dehydrogenase which was also discovered originally in green leaves[8,9]. The properties and intracellular distribution of the various dehydrogenases will be described in another communication[10].

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Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment

Quantitative analysis after starch-gel electrophoresis is usually difficult due to : (1) the diffusion of protein fractions, (2) the difference of migration according to the thickness of the gel, and (3) the fragility and opalescence of this migrating medium.

The cutting of the gels accompanied by superficial staining of the surfaces only partially resolved these difficulties. Some progress has been obtained by cutting thin slices in the median zone of the gel, which can be stained and rendered transparent after inclusion in agar and dried by heating[1,2]. On the other hand, immersion in pure glycerol[3] or transformation into 'plastic' by heat only[4] has been tried. However, the results obtained did not permit a true quantitative analysis. The technique described here resolves these problems: (1) the stain penetrates into the gel completely, (2) the gel is transformed into a thin, transparent and supple film.

Smithies's technique[5] is used for the preparation of the starch-gel. The gel is poured into the apparatus giving a homogeneous layer 3 mm. thick[7]. The lateral diffusion of the more concentrated proteins (for example, albumin) is limited by cutting a thin band (2 mm.) of gel between the samples. A sheet of polyethylene protects the gel from the air. The introduction of the sample is done by mixing the serum in some gel in order to obtain a continuous medium.

With this technique, the serum components give strictly parallel bands. After electrophoresis, there are two possible treatments :

(1) It is possible to transform the gel into plastic before staining. The gel is placed for 1 hr. in a bath of an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. It is then heated with an infra-red tube of 1,200 W. for 3 hr. at a distance of 65 cm. The plastified and transparent plate is stained in aqueous dye solution during 2 hr. (naphthalene black 0·5 gm., glacial acetic acid 50 ml., distilled water 1,000 ml.). Decoloration is obtained by successive washings in 5 per cent aqueous acetic acid solution. The last bath contains 50 per cent glycerol. The gel is dried with filter paper and is heated by an infra-red lamp for 20 min.

(2) It is also possible to stain the gel directly for 4 hr., then after successive washings it is placed for 1 hr. in an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. The gel is heated for 3 hr. with infra-red on a glass plate.

The first technique reduces the risk of deterioration during manipulation. The second results in a more supple plastic film. The transparent band can be measured by direct photometry (Fig 1).

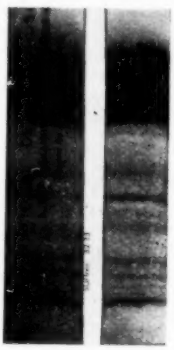

Fig. 1

In addition we noted that Poulik discontinuous buffer system[6] has the advantage of giving a narrow zone (1 cm. wide) of albumin and clearer pre- and post-albumin zones.

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Changes in Glucose-6-phosphatase Activity in Liver and Kidney at Birth

Glucose-6-phosphatase catalyses the last step in the chain of reactions by which glycogen is converted to glucose. The enzyme is found only in liver, kidney and intestinal mucosa. Its activity is low in fœtal liver and increases at birth in the guinea pig[1] and the rat[2]. Glucose-6-phosphatase has been measured in human liver only in the first half of gestation. Cori and Schulman[3] found very low levels in an 18-week fœtus. Villee[4] used the production of glucose by liver slices as a measure of glucose-6-phosphatase. Activity appeared about the tenth week of gestation and had reached about half adult levels by 22 wk. Aurichio

Table 1. CHANGES IN GLUCOSE-6-PHOSPHATASE ACTIVITY AT BIRTH

Species		Fœtus at term	24 hr. after birth	Adult
Rat	Liver	4·9	68·6	20·7
	Kidney	3·3	8·6	26·2
Rabbit	Liver	7·9	46·4	16·0
	Kidney	5·3	8·8	16·8
Guinea pig	Liver	3·4	38·6	16·7
	Kidney	6·0	22·4	17·3
Sheep	Liver	5·7	45·7	20·9
	Kidney	6·0	31·4	24·1

Results are expressed in μM glucose-6-phosphate hydrolysed per mgm. N/hr.; each figure is the mean of at least three determinations done in duplicate.

System: Glucose-6-phosphate, 0·02 M; *tris* buffer pH 7·0, 0·1 M; enzyme 0·1 ml. of a 10 per cent homogenate in 0·25 M sucrose. Final volume 0·5 ml. Substrate was omitted from the blank. Incubated for 10 min. at 37° and de-proteinized with 1 ml. 10 per cent (w/v) trichloracetic acid. Inorganic phosphate liberated was determined by the method in ref. 10.

and Rigillo[5] found low, but variable, activity in post-mortem material from 20 to 40 weeks of gestation.

In the rat, activity increases in the first 24 hr. after birth to levels several times those in adult liver[6,7]. Glucose-6-phosphatase activity has now been measured in liver and kidney in a number of species of differing maturity at birth.

Table 1 shows glucose-6-phosphatase activity in liver and kidney of the fœtus at term, the new-born animal 24 hr. after birth and the mother in four different species. The rat is very immature at birth, with closed eyes and no hair. The new-born rabbit is more mature than the rat, but less mature than the lamb and guinea pig which are born with eyes open and able to run about within a short time of birth. Nevertheless, in all these species there is a considerable increase in glucose-6-phosphatase activity in the liver just after birth. Kidney glucose-6-phosphatase behaves rather differently. In the rat and rabbit there was only a small increase at birth and adult activity was reached 2 weeks after birth. In the lamb and guinea pig, however, activity reached adult-levels within 24 hr. after birth. This species difference might be related to differences in the maturity of renal function at birth.

The effect of delivery on the increase in activity of glucose-6-phosphatase is shown in Fig. 1. The same rise in activity was found in animals delivered at term naturally and by Cæsarean section. Animals delivered two days before term by Cæsarean section showed a rise within 24 hr. to levels as high as those in 24 hr.-old rabbits delivered at term. When pregnancy was prolonged with chorionic gonadotrophin[8], there was no increase until after delivery, when a rise again occurred within 24 hr.

At the end of gestation large amounts of glycogen have accumulated in the liver in all species which have been examined[9]. One possible factor may be low glucose-6-phosphatase activity, as originally suggested[1]. Immediately after birth, liver glycogen disappears very rapidly, presumably to maintain blood glucose in the new-born animal. At this time, there is a very considerable increase in glucose-6-phosphatase activity in the liver in all species which have been examined. This increase in activity is not related to maturity at birth. The effects of early and late delivery suggest that the stimulus for the increase in activity is related to delivery. Nemeth[8] has described similar findings on the effect of delivery on tryptophan pyrollase activity in rabbit liver.

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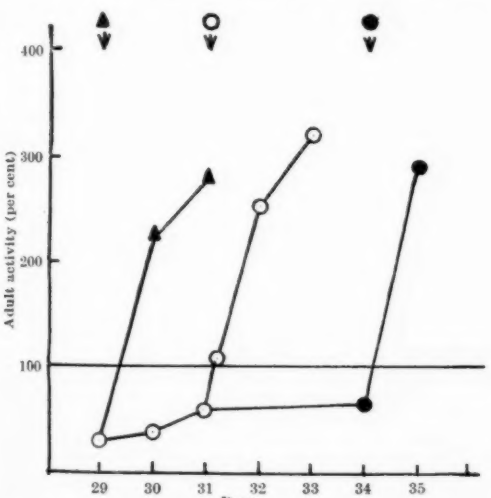

Fig. 1. The effect of premature and postmature delivery on liver glucose-6-phosphatase activity in the new-born rabbit. Results are expressed as a percentage of adult activity (16·0 μM glucose-6-phosphate hydrolysed/mgm. N/hr.). Mature, ○; premature, ▲; postmature, ●. Delivery is indicated by the arrow

Keto-Acids and Amines in Rumen Liquid of Cattle

AN investigation was made into the occurrence of keto-acids in rumen liquid of cattle; simultaneously the amino-acids and the amines were examined, as electrophoresis at pH 4·5 revealed that basic components occurred in rumen liquid. The following methods were used:

Keto-acids: After adjustment of the pH to 4·7 and a treatment with 'Norit', rumen liquid is passed through a 'Dowex 50W' column in order to remove the amino-acids. As mentioned in an earlier communication[1] concerning keto-acids in serum and urine of cattle, the keto-acids are converted into 2,4-dinitrophenyl hydrazones, after which they are reduced by hydrogen to amino-acids and identified as such.

Amino-acids: From the 'Dowex 50W' column the amino-acids are eluted with 6 N ammonia. After evaporation to dryness, the residue is submitted to electrophoresis with evaporation at pH 4·5. In this way a good separation in groups up to and including the amines is obtained. The amino-acids are detected by ninhydrin and copper nitrate solution. The red-coloured spots are eluted with methanol and the extinction is measured at 525 mμ. This method is semi-quantitative, but it gives sufficient information about the changes in the concentrations.

Amines: The pH of a suitable quantity of rumen liquid, for example 40 ml., is adjusted to 4·5, after

which the liquid is centrifuged. Then sodium hydroxide solution is added and a mixture of salts (sodium sulphate and disodium hydrogen phosphate[2]). The *p*H must be ± 12. The liquid is first extracted with ether in order to remove lipids and other interfering material and then three times with butanol. The butanol solution is concentrated *in vacuo* to a small volume and then submitted to electrophoresis, combined with evaporation at *p*H 11·7. Afterwards the paper (27 × 55 cm.) is chromatographed in propanol/0·2 *N* ammonia (3 : 1). From Fig. 1 it can be seen that a good separation even of cadaverine and putrescine is obtained.

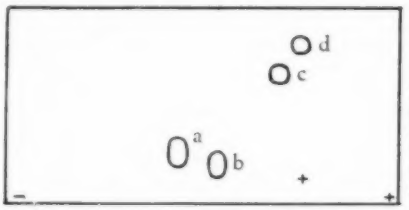

Fig. 1. Separation of amines in the butanol fraction. *a*, Cadaverine; *b*, putrescine; *c*, histamine; *d*, tyramine

In this way the following keto-acids could be shown: pyruvic acid, hydroxypyruvic acid, glyoxilic acid, α-keto-*iso*valeric acid, α-keto-*iso*caproic acid or α-keto β-methylvaleric acid, oxalo-acetic acid and α-keto-glutaric acid. A few components are still under investigation.

It appeared that the concentration of the keto-acids increases within 30 min. after feeding. An examination of hay and grass revealed that these foodstuffs contain keto-acids. Therefore, glucose was added to rumen liquid in order to investigate if keto-acids would be formed. Indeed, it appeared that the concentration of pyruvic acid was now strongly increased. Moreover, it was remarkable that the *p*H decreased noticeably, and that the concentration of the amino-acids and other basic components increased strongly. The fall of the *p*H depends on the time when the sample is taken: shortly after feeding, the decrease of *p*H, after addition of glucose, is at its greatest.

As it was possible that the basic components contain amines, for example, histamine and tyramine, which Dain *et al.*[3] identified in rumen liquid of experimentally over-fed sheep, an investigation in this direction was carried out.

In a first experiment glucose, histidine, tyrosine and tryptophan were added to rumen liquid in order to examine if decarboxylation occurred. Indeed, histamine, tyramine and probably tryptamine could be detected after 24 hr. Afterwards, rumen liquid, to which fodder-cakes, caseine and glucose, and caseine alone were added, was examined. Frequently, histamine and tyramine could be detected, but cadaverine, putrescine and colamine occurred in far greater amounts. If lysine were the most important component of the basic amino-acids, the concentration of cadaverine was high; by addition of arginine to rumen liquid the concentration of putrescine increased strongly. From these results it is postulated that lysine in the rumen forms cadaverine and that arginine, after conversion to ornithine, forms putrescine.

The factors responsible for the formation of the amines and amino-acids are under investigation *in vitro* and *in vivo*. In one case, namely, a heifer, a greater concentration of amines was found in the rumen.

I am much indebted to Prof. G. Wagenaar for putting the cows at my disposal and to Mr. C. G. van der Kamp for technical assistance.

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Haptoglobinuria in Nephrotic Syndromes

As a rule, only traces of haptoglobin can be found, by titrimetric method, in the urine of patients presenting a nephrotic syndrome with considerable proteinuria, as against their serum, which is very rich in α_2 globulin and especially in haptoglobin. Actually, it is so only in the case of patients belonging to Smithies's groups *2·1* and *2·2* (ref. 1), for, when the serum belongs to group *1·1*, the haptoglobin, of lower molecular weight, filtered through the nephrotic kidney, can reach a concentration of several gm. per litre[2,3].

I have had the opportunity of examining the urines of patients whose haptoglobin was type *2·2* in some, while in others it was type *2·1*.

In the urines of patients with haptoglobin of type *2·2*, I could never find any haptoglobin either by Jayle's titrimetric method (measure of the peroxidasic activity of the hæmoglobin–haptoglobin complex), or by electrophoresis in starch-gel, even when the haptoglobin was very much increased and the proteinuria very considerable. For example, in the case of patients with renal amyloidosis, the serum haptoglobin was as high as 10 gm./l., the proteinuria was 20 gm./l.; the urine, very rich in seromucoid α_1, contained no haptoglobin. The same result was found in the case of lipoidic nephrosis belonging to Smithies's group *2·2*, and this, whatever the rate of haptoglobin in the serum.

With patients having haptoglobin of group *2·1*, the titrimetric method has always allowed detection of a small quantity of haptoglobin in the urine, either directly if the proteinuria were important, or by concentrating the urine after dialysis if the proteinuria were rather low. For example, in the case of a patient whose serum haptoglobin remained about 5 gm./l., 0·50 gm. of haptoglobin was found in the urine for a proteinuria of 25 gm./l. When the latter was 7 gm., there was still 0·10 gm. of haptoglobin; when it went down to 0·20 gm./l., I still could, by sufficient concentration of the urine, reveal a small quantity of haptoglobin. In other patients with haptoglobin of the same group *2·1* and haptoglobinæmia of 4·5 gm./l., but with a comparatively low proteinuria (about 2 gm./l.), I found, by concentrating the urine ten times, a very small but still measurable quantity of haptoglobin.

The urine of all these patients was analysed by electrophoresis in starch-gel following Smithies's method, after preliminary addition of hæmoglobin so as to reveal the hæmoglobin–haptoglobin complexes, very small quantities of which can be detected by benzidine staining.

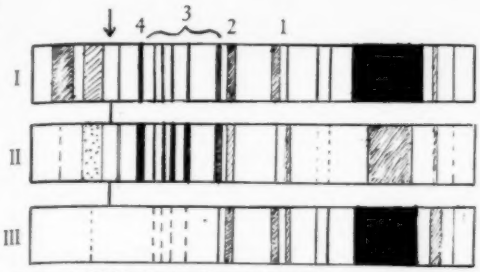

Fig. 1. Amidoblack coloration. I, Normal serum; II, nephrotic serum; III, nephrotic urine. (1) Haptoglobin *1·1*.; (2) β globulin *C*; (3) haptoglobin zone; (4) slow $\alpha 2$

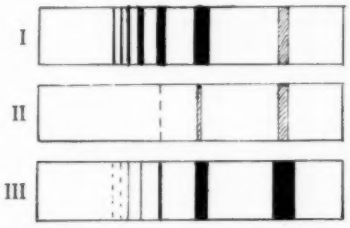

Fig. 2. Haptoglobin *2·1* hæmoglobin complex in nephrotic syndrome; benzidine coloration. I, Serum; II, urine; III, haptoglobin obtained from urine, by saline precipitation

When proteinuria is very low, the urines are concentrated before including them in the starch-gel, or the haptoglobin can also be isolated by salt precipitation.

Results: (*a*) Homozygous patients with haptoglobin *2·2* have no haptoglobin in their urine. (*b*) Heterozygous patients with haptoglobin *2·1* have three distinct bands in their urine. The first corresponds to haptoglobin *1·1*, the second to haptoglobin β and the third to the most anodic band of the $\alpha\beta$ haptoglobins (haptoglobin *1·1* and β do not occur in sera of group *2·2*) (Figs. 1 and 2).

It is to be noted that the intensity of haptoglobin *1·1* is the same as in the serum, while that of the following band is much weaker than in the serum. The pattern of urinary haptoglobin is identical, whether the concentration of haptoglobin in the urine is 0·1 gm. or 1 gm./l.

In the case of a patient suffering from renal amyloidosis and whose proteinuria was very high, we separated the urinary haptoglobin by salt precipitation and I obtained 0·8 gm. per 100 ml. solution. The starch-gel electrophoresis pattern showed all the bands of the serum, but their proportion varied greatly in the urine and the serum. A very large quantity of haptoglobin *1·1* and very small quantities of the least anodic components were found. The β component is less considerable than the haptoglobin *1·1*; in serum, on the other hand, it is much more intense.

Table 1

Haptoglobin type	Serum haptoglobin (gm./l.)	Proteinuria (gm./l.)	Urinary haptoglobin (gm./l.)	
			Directly titrated	After concentration
La 2·1	4·9	25	0·50	
	5·5	7	0·10	
	5·0	6		0·60
	5·0	2		0·12
	5·2	1·5		0·10
	5·0	0·2		0·10
B 2·1	4·0	2		0·10
	4·2	2·3		0·14
	5·0	1		0·05
	5·0	0·5		traces
Le 2·1	5·0	20	0·10	
	5·0	24	0·10	
	5·5	18	0·08	
R 2·2	10·0	20	0·00	
Ca 2·2	4·6	24	0·00	
	5·0	8	0·00	0·00

These results confirm that the haptoglobin zones represent distinct protein components, and confirm the difference between the molecular size of these haptoglobins. I find mostly, in nephrotic urine, haptoglobins the molecular weight of which is relatively low and which belong to type *2·1* and not to type *2·2*.

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A New Triterpenoid Sapogenin of Animal Origin (Sea Cucumber)

In earlier papers one of us (T. Y.)[1] reported the isolation, chemical properties, and toxicity to fish and warm-blooded animals, of crystalline saponin from the body wall of the sea cucumber, *Holothuria vagabunda*, and designated this as a new saponin, holothurin.

A similar saponin also named holothurin was independently isolated by Nigrelli *et al.*[2] from *Actinopyga agassizi*, a sea cucumber found in the Bahama Islands. Recently, by the extensive study of Sobotka *et al.*[3], holothurin was shown to be further separable into two components: precipitable and non-precipitable by cholesterol. The former component was designated holothurin *A* and proved to be a mixture of several glycosides each of which contains a steroid aglycone of about 26–28 carbon and 4–5 oxygen atoms, one molecule each of four different sugars and one molecule of sulphuric acid as the sodium salt. As sugar components, glucose, xylose, glucomethylose and 3-O-methyl glucose were identified.

Naturally occurring saponins can be divided into two groups. The first of these gives steroidal sapogenins on hydrolysis, the second triterpenoid sapogenins. Triterpenoid sapogenins, so far as we know, have been isolated only from the plant kingdom. It is, therefore, of considerable biogenetic interest to report in this communication the isolation of a triterpenoid sapogenin of animal origin, sea cucumber.

The main sapogenin which was obtained by acid hydrolysis of holothurin was found to be $C_{30}H_{44}O_5$ (I), m.p. 301°, $[\alpha]_D$ + 14·9°, giving a brown coloration with tetranitromethane. It appeared to be an unknown triterpene and was named holothurigenin. Both holothurin and holothurigenin showed an infrared absorption band at 1,767 cm.$^{-1}$ attributable to a five-membered lactone. This would indicate that the lactone ring was present in the animal material rather than produced during the acid hydrolysis of the saponin. The direct proof for its presence was

provided by the course of the lithium aluminium hydride reduction of holothurigenin (I), which led to the corresponding diol (II), $C_{30}H_{48}O_5$, m.p. 223°, $[\alpha]_D$ − 62·8°, $\lambda_{max.}$ 244 mμ, log ε 4·22, infra-red: 3,448, 3,509, and 3,636 cm.$^{-1}$.

On mild acetylation with acetic anhydride–pyridine of (I), monoacetate (III), $C_{32}H_{46}O_6$, m.p. 277–8°, $[\alpha]_D$ − 10·3°, $\lambda_{max.}$ 244 mμ, log ε 4·42, infra-red: 1,724, 1,767, and 3,636 cm.$^{-1}$ was obtained, which was unaffected by chromic acid/pyridine. Chromic acid/pyridine oxidation of (I) gave a monoketone (IV), $C_{30}H_{42}O_5$, m.p. 290–1°, $[\alpha]_D$ −40, $\lambda_{max.}$ 244 mμ, log ε 4·28, $\lambda_{max.}$ 285 mμ, log ε 2·65, infra-red: 1,701, 1,767, and 3,636 cm.$^{-1}$, which was identical with monoketone obtained by chromic acid oxidation (equivalent to one oxygen atom) in acetic acid solution of (I) at room temperature. Drastic acetylation of (I) led to the corresponding triacetate (V), $C_{36}H_{50}O_8$, m.p. 248°, $[\alpha]_D$ −26·4, infra-red: 1,724, 1,736, and 1,764 cm.$^{-1}$. (V) was reconverted into the parent triterpene (I) on saponification.

Thus the five oxygen atoms were demonstrated to be present as five-membered lactone ring and three hydroxyl groups which differed in their reactivity. It was clear that two of three hydroxyl groups are oriented in hindered positions.

The ultra-violet absorption of (I) exhibited a primary maximum at 244 mμ (log ε 4·15), with subsidiary maxima at 238 and 255 mμ. Coupled with the colour reaction with tetranitro-methane of (I), this suggested the presence of a conjugated hetero-annular diene. By catalytic hydrogenation in acetic acid solution of (I), dihydroholothurigenin (VI), $C_{30}H_{46}O_5$, m.p. 285°, $\lambda_{max.}$ 215 mμ, log ε 3·01, was obtained in which the ultra-violet absorption band observed at 244 mμ had disappeared due to a conjugated double bond.

At this stage of the investigation it was concluded that holothurigenin was a trihydroxy-lactone-diene belonging to the triterpenoid series. The structure of holothurigenin is now being investigated.

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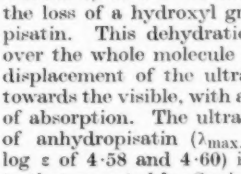
Pisatin: an Antifungal Substance from *Pisum sativum* L.

It is obvious that plants are resistant to attack by fungi that are non-pathogenic to them. However, until recently, the mechanism of this resistance has been obscure. Pisatin, a substance believed to be responsible for such resistance in the garden pea (*Pisum sativum* L.), has been recently isolated from pea pods inoculated with fungal spores[1].

Pisatin has the molecular formula $C_{17}H_{14}O_6$, and, from chemical and spectroscopic evidence, contains one alcoholic hydroxyl, one methoxyl and one methylenedioxy group. Detailed examination of pisatin and some of its degradation products by physico-chemical methods, including nuclear magnetic resonance, ultra-violet and infra-red spectra, indicates that pisatin has structure I.

The location of the methoxyl and methylenedioxy groups is suggested by the very close similarity of the ultra-violet and infra-red spectra to those of pterocarpin[2], although the possibility that the methylenedioxy group is attached to different positions in the same ring cannot be entirely excluded. Pisatin has ultra-violet absorption maxima at 309 mμ (log ε 3·86) and 286 mμ (log ε 3·68) with an inflexion at 281 mμ, while pterocarpin has maxima at 310 mμ (log ε 3·92) and 286 mμ (log ε 3·69) with an inflexion near 280 mμ. Pisatin is optically active with $(\alpha)^{20°}_{578} = +280$ (c = 0·11 in ethanol). Pterocarpin has comparable optical activity, but is lævorotatory[2]. Pisatin appears to differ from pterocarpin only in having a hydroxyl group on carbon 3, and also by possibly having the opposite optical configuration.

The presence of the hydroxyl group confers some interesting chemical properties on pisatin. Thus in the presence of acid, pisatin readily loses a molecule of water across positions 3 and 4 to form anhydropisatin, $C_{17}H_{12}O_5$ (ref. 1). The disappearance of the band at 3,610 cm.$^{-1}$ in the infra-red spectrum confirms the loss of a hydroxyl group in passing to anhydropisatin. This dehydration extends the conjugation over the whole molecule and leads to a considerable displacement of the ultra-violet absorption maxima towards the visible, with a concomitant intensification of absorption. The ultra-violet absorption spectrum of anhydropisatin ($\lambda_{max.}$ at 339 mμ and 358 mμ; log ε of 4·58 and 4·60) is qualitatively very similar to that reported by Suginome[3] for the closely related substance, anhydrosophorol, formed by cyclodehydration of sophorol, a 2′-hydroxy isoflavanone obtained from *Sophora japonica* L. (Leguminosæ, sub-family Papilionaceae).



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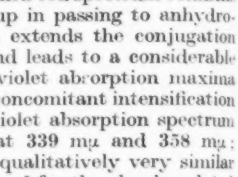

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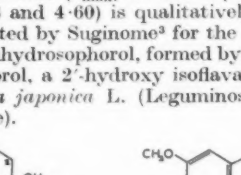

III

When alcoholic solutions of anhydropisatin are heated or exposed to diffuse daylight, fission occurs across the 1 : 2 oxygen–carbon bond giving rise to compound (II). The ultra-violet spectrum of this substance with $\lambda_{max.}$ 269 mμ (log ε 4·18) and 319 mμ (log ε 4·39) is similar to those reported for related 2-phenyl benzofurans[4]. On the other hand, if pisatin itself is irradiated with mercury 253·7 mμ light, it is converted initially to a substance which is spectroscopically very similar to pisatin. This substance differs from pisatin, however, in not forming anhydropisatin on mild acid treatment, and in not showing comparable antifungal activity[5]. Further irradiation followed by acidification gives rise to the isoflavylium compound (III) which is cherry red in strong acid and greenish yellow in weakly acid or neutral solutions (cation has $\lambda_{max.}$ 419, 552 mμ; log ε 4·0, 4·0; neutral molecule has only $\lambda_{max.}$ 419 mμ, log ε 4·0). Spectrophotometric examination shows that this compound is half converted to the cation in 3·4 *M*

hydrochloric acid, which indicates that this base is about 100,000 times weaker than the flavylium bases such as anthocyanidins. Compound (III) has similar properties to those reported for the isoflavylium compound prepared by Baker[6].

The ring structure of pisatin occurs in a number of other natural products including pterocarpin and homopterocarpin[2] (from *Pterocarpus santalinus* L.f.), coumestrol[7] (from ladino clover *Trifolium repens* L.), erosnin[8] (from the yam bean *Pachyrrhizus erosus* Rich.), psoralen[9] (from *Psoralea corylifolia* L.) and wedelolactone[10] (from *Wedelia calendulacea* Less.). It is of interest that in all cases, except *Wedelia calendulacea* (Compositae), the plants, from which this relatively new series of compounds have been obtained, are members of the family Leguminosae (Papilionaceae).

Further details of this work will be published elsewhere.

We wish to thank Mr. B. Moore and Prof. A. J. Birch, of the University of Manchester, for a sample of pterocarpin.

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Prodigiosin in an Actinomycete

PRODIGIOSIN, the bright red pigment produced by organisms of the genus *Serratia*, is among the more conspicuous pigments extant in the microbial world. The chemical nature of prodigiosin has been the subject of extensive study and was defined as a tripyrrylmethene by Wrede and Rothhaas in 1934[1]. Subsequent work has demonstrated that the pigment is actually composed of a number of separable components rather than the single entity supposed by earlier workers[1]. Although prodigiosin has been studied predominantly in the genus *Serratia*, the possible presence of the pigment in an actinomycete has been reported[2]. In the course of our isolating vast quantities of actinomycetes from soils collected throughout the world, a small number of orange-red pigmented *Streptomyces* were observed. This communication is concerned with the identification of the pigment produced by these actinomycetes as identical to that produced by *Serratia marcescens*.

The actinomycetes were grown on a medium consisting of soytone, casein hydrolysate, and yeast extract at 0·1 and 3 per cent agar. Plates were inoculated from slants of the actinomycete. After growth for 5 days at 28° C. the cells were washed from the agar surface with acetone/water (50 : 50), dried under vacuum, and extracted with ethanol in a Soxhlet extractor. The pigment was further purified according to the procedure of Williams *et al.*[3].

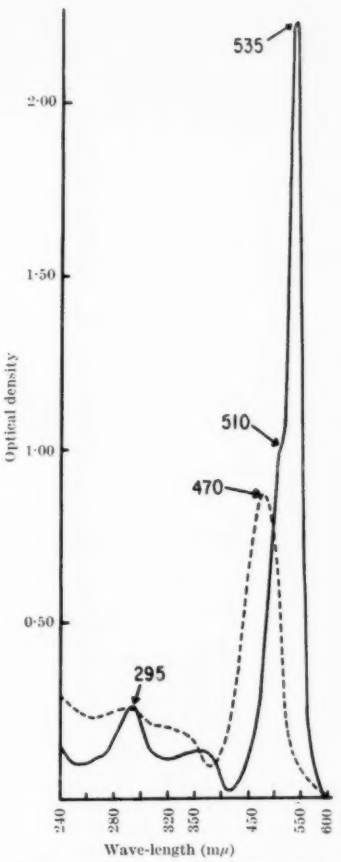

Fig. 1. Spectral properties of purified prodigiosin from an actinomycete. Solid line, when dissolved in absolute ethanol + hydrochloric acid. Broken line, in absolute ethanol + sodium hydroxide

Chromatographic separation of the prodigiosin components was achieved by ascending chromatography using Whatman No. 1 paper and developed with petroleum ether/ethyl ether (2 : 1). Comparison of the chromatographic behaviour of the actinomycete pigment with the bacterial pigment[3] indicated that some differences in the relative quantities of the various components between the two species do exist. The extracts from both actinomycete and *S. marcescens* gave orange-red zones at the following R_F values : 0·92, 0·85, 0·74, 0·46, and a blue spot at the origin.

For the purpose of obtaining ultra-violet and visible spectra of the actinomycete pigment, approximately 100 μgm. of dried pigment was chromatographed. After development, the coloured area at R_F 0·92 was eluted with 10 ml. ethanol. This was the predominant spot on the paper. For a control an equal area of blank paper at the same R_F was also eluted. Spectral analyses were run on a Cary recording spectrophotometer. Acid or alkaline conditions were obtained by adding 1 drop of 1 *N* hydrochloric acid or 1 *N* sodium hydroxide to the ethanol extract.

The acid and alkaline spectra of the pigment are presented in Fig. 1. The alkaline spectrum has a peak at 470 mμ. The acid spectrum exhibits peaks at 535 mμ and 295 mμ and a shoulder at 510 mμ. These results are in agreement with published spectra for bacterial prodigiosin[3]. Chromatographic comparison of the bacterial and actinomycete pigment in various solvents[4] showed that the same components were present in each although the relative amounts varied.

The results demonstrate that actinomycetes of the genus *Streptomyces* producing prodigiosin are present in soils. Prodigiosin was isolated, and is qualitatively equivalent to the bacterial pigment, although differences in relative quantities of the components do exist.

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PHYSIOLOGY

Vitamin-Levels in Blood and Serum

Except for folic acid and cyanocobalamin (vitamin B_{12}) only scattered information can be found in the literature on the content of water-soluble vitamins in human blood and serum[1,2]. In order to establish basic values of normal blood and serum vitamin for various clinical investigations, microbiological assays were carried out for a number of vitamins by the methods which we have developed.

Blood was obtained from an antecubital vein and permitted to clot at room temperature for 3 hr. After centrifugation, serum was withdrawn for assay. Care was taken to obtain serum free of any hæmolysis; intracellular contents gave erroneous values. Citrated blood was used for whole-blood assays. Blood and serum were assayed for (*a*) B_{12} with *Ochromonas malhamensis*[3a], (*b*) folic acid with *Lactobacillus casei*[3b], (*c*) unconjugated pteridines with *Crithidia fasiculata*[3c], (*d*) thiamine with *O. malhamensis*[3d], (*e*) nicotinic acid with *Tetrahymena pyriformis*[3f], (*f*) pantothenic acid with *L. plantarum*[3e], and (*g*) vitamin B_6 with *T. pyriformis*[3e]. Table 1 gives the range encountered both in whole blood and in serum of 28 normal subjects. The whole-blood values for folic acid, unconjugated pteridine, thiamine, nicotinic acid, and perhaps for the B_6 group (Table 1) are higher than those for serum; the reverse is true for B_{12}. Nicotinic acid activity almost wholly resides in the cellular elements of the blood.

The folic acid activity of serum, not whole blood, serves as an index of clinical folic acid deficiency[3b]. In blood of normal subjects the cells carry 1/5–1/6 the total B_{12}; in pernicious anæmia their share is 1/2–1/3[4a,b]. Serum B_{12}-levels reflect the tissue supply.

Table 1. Vitamin Activities of Whole Blood and Serum (μgm./ml.)*

Vitamin	Whole blood	Serum
B_{12}	0·12–0·45	0·14–0·64
Folic acid	14–72	7–27
Unconjugated pteridine	27–70	11–43
Thiamine	20–41	3–15
Nicotinic acid	4,900–9,600	50–80
Pantothenic acid	230–2,075	200–1,650
B_6 group	26–78	26–43

* Based on 28 normal subjects.

In other work, we have observed exceptionally high levels of unconjugated pteridines in sera from patients with gout, while folic acid activity was in the normal range. The normal values presented here have also served as controls in comparative work on the vitamin content in maternal versus fœtal blood[5a,b] and for comparison with the cerebrospinal fluid values in patients with neurologic disease[6a,b].

We thank Dr. T'sai F. Yu of the Department of Medicine, Mount Sinai Hospital, for supplying samples from patients with gout; also we thank the U.S. Public Health Service, the National Association for Mental Health, and the American Cancer Society for financial support.

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Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude

It has been shown[1] that during a prolonged stay at altitudes above 15,000 ft. there is an increase in the salivary excretion of sodium and a decrease in that of potassium. In one subject this change in salivary electrolyte composition showed a marked acclimatization effect (Fig. 1). These findings suggested that, on arrival at a high altitude, the level of circulating aldosterone was likely to be greatly reduced, but that there would probably be a tendency for it to return slowly towards normal values with passage of time.

During July and August 1960, members of the Middlesex Hospital Medical School High Altitude Physiological Expedition spent twenty-four days at an altitude of 14,300 ft. on Mont Blanc[2]. 24-hr. urine collections were made from seven subjects (four men and three women). These specimens were frozen and sent in 'Styrocell' insulated containers by air to London, where they arrived still solid.

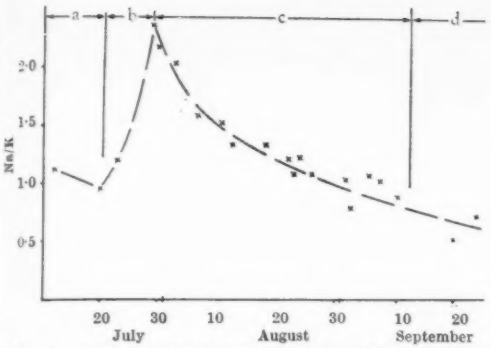

Fig. 1. Salivary sodium/potassium in one individual during an expedition to the Karakoram Mountains. – –, sodium/potassium level in London; *a*, 8,000–10,000 ft; *b*, 10,000–16,000 ft.; *c*, 16,000–20,000 ft.; *d*, below 10,000 ft. Each point is derived from five observations

Assay of urinary aldosterone was carried out by a physico-chemical method modified from that of Ayres *et al.*[3]. The sodium and potassium contents were determined by flame photometry. The results are shown in Fig. 2 and it will be seen that the inference from the earlier work is correct, assuming the validity of urinary aldosterone assay as an index of circulating hormone-levels.

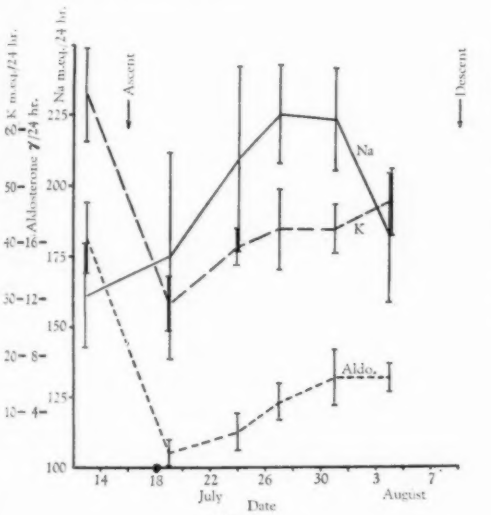

Fig. 2

The decreased potassium excretion apparent from Fig. 2 should result in a demonstrable increase in body potassium content. An attempt was made by one of us (J. R.) to confirm this in three subjects, whose whole body potassium contents were estimated by taking advantage of the naturally radioactive isotope potassium-40, present in potassium to the extent of 0·0119 per cent. The body radioactivity was measured in the human gamma-ray spectrometer[4] at the Atomic Energy Research Establishment, Harwell, before the expedition, and again two days after the descent from the mountain. The results are shown in Table 1. At each measurement the potassium content was calculated from the counting-rates observed in the energy bands 875–1,275 keV. and 1,275–1,675 keV. in the gamma-ray spectrum (potassium-40 emits 1·46 MeV. quanta). The equipment was calibrated by determining the counting-rates in these energy bands from known amounts of pure potassium chloride in aqueous solution in a phantom, and there may be systematic errors of up to about ± 5 per cent in the estimates of the contents of individuals[4]. In all cases the agreement between the two estimates of each subject's content was within the statistical error of counting, indicating the absence of detectable contamination with other radioactive nuclides emitting gamma-rays of energy greater than 0·8 MeV. The values in Table 1 are the weighted means of the pairs of estimates, with statistical standard errors only.

Table 1

Subject	Total potassium content (gm.)		
	Before expedition	After descent	Difference
P. M. ♀	107 ± 3·8	115 ± 3·8	8 ± 5·4
E. W. ♂	146 ± 4·0	152 ± 3·9	6 ± 5·6
W. W. ♀	100 ± 4·2	110 ± 3·8	10 ± 5·7
Total	353 ± 6·9	377 ± 6·6	24 ± 9·6

In only one subject is the increase in potassium content significant at the 90 per cent confidence-level, but the increase in the total content of all three subjects is significant at the 99 per cent confidence-level.

During the stay on the mountain the diet of the subjects consisted largely of dehydrated foodstuffs. In order to establish what effect the diet had on the results obtained, one of the subjects carried out a control experiment at sea-level. 24-hr. urine samples were collected for five consecutive days while he was on a normal diet, and then continued while he was on the same diet as he had eaten on the mountain. These were assayed for sodium and potassium, and three of the five samples relating to the period of normal diet and five of those relating to the period on the special diet were assayed for aldosterone.

The aldosterone results are plotted in Fig. 3 where they are compared with the results from the same subject for the corresponding period on Mont Blanc. It will be observed that a drop in aldosterone

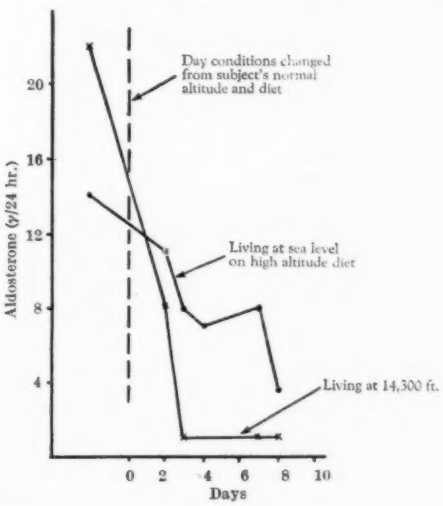

Fig. 3

excretion followed the change in diet, but that this drop was less marked than it was when a change in altitude coincided with the dietary change. If the mean 24-hr. excretion before and after the changed conditions is compared in each case, we see that on the mountain the excretion dropped to 12·5 per cent of its previous mean whereas it dropped to 53·5 per cent when the diet alone was changed.

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Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation

It has long been suggested that there exists a relationship between colloidal behaviour of muscle and that of blood, namely, (1) molecular structure of actomyosin and that of fibrinogen (both belong to the *k–m–e–f* group[1]), (2) coagulative phenomena both in sarcoplasm and in blood[2], and (3) the role of calcium both in contractile activity of muscle and in blood coagulation[3]. Recently, Heilbrunn[2] has suggested that the mechanism of blood coagulation in relation to the mechanism of cellular activity may be significant. The present communication is concerned with the positive results, which were obtained in a model system of the muscle, to demonstrate the close resemblance between the mechanism in mechanical muscular contraction and that in blood coagulation.

As shown in Fig. 1, phospholipids remove the inhibitory effect[3] of muscle granules on adenosine triphosphatase activity of myofibrils prepared from skeletal muscle. The concentration at which the phospholipid effect begins to appear is relatively low. In Fig. 2, it is shown that heparin inhibits the adenosine triphosphatase activity of myofibrils prepared from skeletal and cardiac muscle. The inhibition is not complete, even if considerably higher concentrations of the agent are used, while the effect begins to appear at relatively lower concentrations, especially in the case of skeletal myofibrils. It is interesting that in the case of myofibrils prepared from heart, which is poor in heparin[2], the inhibitory effect is relatively lower, for example, at 100 units/ml. of heparin it is only about 50 per cent; while, under the similar condition, it is considerably higher in the case of skeletal muscle, which is rich in heparin[2]. The development of tension of a single glycerinated fibre of rabbit psoas muscle due to adenosine triphosphate is also inhibited by the addition of heparin. One more interesting point is that adenosine triphosphatase activity of granules

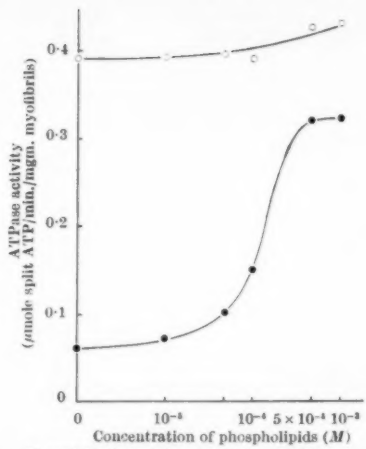

Fig. 1. Phospholipids mixture and the inhibitory action of granules on adenosine triphosphatase activity of myofibrils prepared from rabbit skeletal muscle. ○, Splitting of adenosine triphosphate by fibrils in the absence of granules; ●, that in the presence of granules. The reaction mixture of 3 ml. consisted of 5 m*M* adenosine triphosphate, 5 m*M* magnesium chloride, 5 m*M* potassium oxalate, 360 μgm. protein/ml. of fibrils, 28 μgm. protein/ml. of granules, and 20 m*M* *tris*(hydroxymethylamino)-methane (*p*H 7). Final concentration of potassium in the reaction mixture was 50 m*M*, and the experiments were done at 20° C. Phospholipids mixture used consists of 55 per cent lecithin, 27 per cent colamine-cephalin, 2 per cent serine-cephalin, 5 per cent inositol phosphatid and 5 per cent sphingolipid; and molarity of phospholipids in the reaction solution was calculated as 775 average molecular weight. Concerning myofibrils and granules, see the explanation in Fig. 2

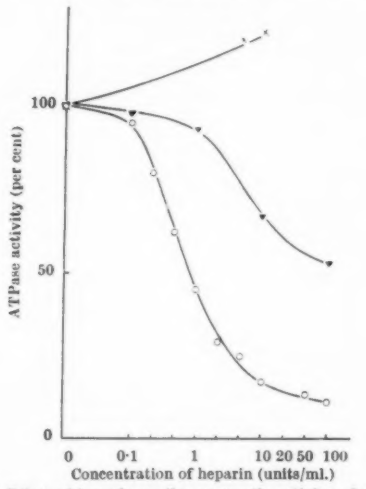

Fig. 2. Effect of heparin on the enzymatic activity of various kinds of adenosine triphosphatase. ○, Myofibrils prepared from rabbit skeletal muscle; ▼, that from dog cardiac muscle; and ×, granules extracted from rabbit skeletal muscle. Myofibrils were obtained according to Perry *et al.* (ref. 8); and granules from 2,600*g* to 35,000*g* were extracted from muscle homogenate, which were obtained in the solution, consisting of 0·1 *M* potassium chloride, 5 m*M* potassium oxalate, and 5 m*M* phosphate buffer (*p*H 7). Conditions of experiments are similar to those of Fig. 1

prepared from rabbit skeletal muscle is not inhibited by heparin. The inhibitory action of heparin is therefore specific to the kind of adenosine triphosphatase. The inhibitory action cannot be removed by the addition of calcium and appears regardless of concentrations of adenosine triphosphate.

It is well known that muscle contains various kinds of phospholipids, especially cephalin and lecithin, and

heparin[2]. However, their functions are not always clear. Of course, our results are not direct evidence of their significance in the mechanical activity of muscle and do not indicate the dual relationship between the mechanical activity of muscle and blood coagulation, with respect to the processes on which the substances mentioned have an effect. Our results are, however, considered to be very interesting in that substances which play an important part in blood coagulation exert some influence on the function of the contractile element of muscle in the same way as blood coagulation. In view of the liberation of heparin-like substances[3] from skeletal muscle during continued activity and of the existence of lipids[4] in microsome or sarcoplasmic reticulum, it would seem probable from our results that a certain lipid compound, especially a substance related to phospholipids such as cephalin and lecithin, and a certain substance related to heparin, control the activity of the contractile element of muscle. (Our preliminary experiments show that the fatigue substance[5] from frog leg muscles has an inhibitory action on the development of tension of the above fibre due to ATP.)

Many reports[5–7] concerning the mechanism in the excitation-contraction link have been presented. The conclusive solution for this problem has, however, not yet been given. Our results suggest the complexity of the linking mechanism and the resemblance of this mechanism to the mechanism in blood coagulation.

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Increase of Evaporative Loss of Water from the Skin of the Ox in Response to Local Heating of the Hypothalamus

The existence of centres in the hypothalamus which are concerned with thermoregulation has been established for several species of mammals[1]. In the cat and monkey[2,3] local heating of the region between the optic chiasma and the anterior commissure is accompanied by vasodilatation, an increase in respiratory-rate and sweating.

In the ox, heating of a discrete region of the hypothalamus causes panting and vasodilatation[4], but it is not known if such heating causes any change in the rate of vaporization of water from the skin. Nor is it certain that the sweat glands play any part in thermoregulation[5]. The effect of local heating of the hypothalamus of bull calves on vaporization of water from their skin is reported here.

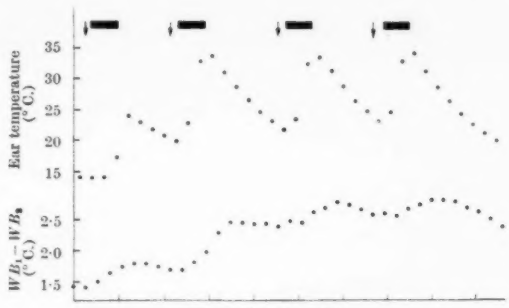

Fig. 1. The figure is taken from a recorder chart on which each temperature was recorded every 80 sec. An increase in the difference between the wet-bulb temperatures ($W.B._1$–$W.B._2$) indicates an increase in evaporation from the skin. The arrows mark the point at which heating of the hypothalamus was started, while the blocks show the 3-min. periods during which the temperature of the hypothalamus was at 41·5° C.

The brain was heated by means of a radio-frequency voltage applied between two electrodes 1·5 cm. apart on either side of the midline, dorsal to the optic chiasma. A thermocouple midway between the electrodes recorded the temperature to which the brain was heated. Moisture loss from the skin was measured by means of a metal 'sweat cup' through which a stream of air was drawn at a constant rate. The cup was applied to the unshaved skin by means of an elastic belt. Comparison of the wet-bulb temperatures of the air entering and leaving the cup revealed changes in the rate of vaporization of moisture from the skin. Skin temperatures were determined with (40 standard wire gauge) copper-constantan thermocouples applied to the shaved skin of the pinnæ of the ears while skin temperatures under the 'sweat cups' were measured beneath the hair. The rate of respiration was recorded by means of an electro-mechanical counter[6].

Three Ayrshire bull calves, all aged one year, were exposed to room temperatures of 10° C.–20° C. in a climatic room. Three 'sweat cups' were disposed over the thoracic, mid flank and sacral regions of each calf. When the temperature of the hypothalamus was raised to 41·5° C. and held there for 3 min. the rate of loss of moisture in each cup increased in 31 out of 35 instances, although the magnitude of the increase varied. In the four experiments in which there was little or no increase in loss of moisture, the rate of evaporation was already high. The response of one animal (*D*) was considerably lower than that of the other two (*B* and *C*). This animal was much smaller and was known from other work to be inapt in the control of its body temperature in a hot environment.

In all three calves, at the same time as evaporation from the skin increased there was a rise in respiratory-rate, and in those instances in which initially the skin temperatures of the ears were low there was a marked rise in the ear temperature indicating increased blood-flow. Small increases in skin temperature under the 'sweat cups' were also noticed on most occasions. As may be seen in Fig. 1 the rate of loss of moisture increased rapidly while the hypothalamus was being heated and continued to rise after heating had ceased; thereafter evaporation

declined slowly. Heating the hypothalamus for less than 3 min. or to less than 41·5° C. produced only a very small increase in evaporation, although a rise in the skin temperature of the ears occurred and respiratory-rate increased.

When the hypothalamus was repeatedly heated to 41·5° C. for 3 min. at intervals, the rate of evaporation from the skin increased progressively (Fig. 1), but the magnitude of the increase tended to decline with successive stimulations.

The results of the present work demonstrate that evaporation from the skin as well as vasodilatation and panting can be stimulated by heating the hypothalamus. It is not, however, suggested that a rise in the temperature of the hypothalamus is a necessary condition for an increase in loss of moisture from the skin.

The increase in evaporation may be related to the active secretion of the sweat glands, or to an increase in diffusion of water through the skin. The progressive increase in evaporation after repeated heating is, however, difficult to account for if evaporative loss is due to simple diffusion.

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Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of Mice

We have reported that œstrogens stimulate the reticulo-endothelial system in the spleen, liver and lymph nodes and lead to mobilization of the phagocytes into the blood stream and thus probably to an increase of the general defence of the body against infection[1]. We have also shown that antibiotics and sulphonamides[2] have little effect on the phagocytes; but cortisone depresses the reticulo-endothelial system and also reduces the concentration of gamma-globulin in the serum to about half the normal level[4]. Unpublished research in this laboratory also shows that when the reticulo-endothelial system is stimulated with, for example, diethylstilbœstrol the serum gamma-globulin is increased to about twice the normal level, and the formation of antibody is increased about eight times. Groups of mice and guinea pigs may be protected by this means against challenge with a number of lethal doses of virulent bacteria. Further unpublished results show that, when animals are given a diet free from vitamin *A*, the reticulo-endothelial system becomes greatly depressed and when normal diet (Oxo mouse and rat diet) is resumed the phagocytic index returns to normal in about thirteen days.

The present research was planned to ascertain the effect of added vitamins on the phagocytic activity of the reticulo-endothelial system in normal healthy animals.

Two hundred and ten male white mice (T.O. Swiss strain) of 20–25 gm. weight were used in these experiments. Ten animals were used to test each vitamin. The vitamins used, the dose, route of administration, frequency and duration of dosage are shown in Table 1. The estimated daily vitamin requirements, also shown in Table 1, are those given by Spector[5].

The doses in most of the experiments are greater than the estimated requirement and during the treatment the animals remained on normal diet. Vitamin D was dissolved in olive oil, vitamin E and vitamin K (natural) in arachis oil to which a small amount of 'Teepol' was added, and the remaining vitamins were given in aqueous solution suitably buffered for stability according to information kindly supplied by Roche Products. All the solutions were made up so that one dose was contained in 0·2 ml. The route

Table 1. Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System

Vitamin used	Estimated daily requirement	Dose	Route	Frequency of dosage	Duration of dosage	Mean phagocytic index (*K* value) ± *S.E.*
Vitamin A palmitate	20 I.U.	70 I.U.	I.P.	2/week	1 week	14 ± 2·0
		70 I.U.	I.P.	2/week	2 weeks	14 ± 4·0
		70 I.U.	I.P.	2/week	3 weeks	12 ± 1·0
Calciferol	2·0 I.U.	10 I.U.	oral	2/week	1 week	11 ± 2·0
		10 I.U.	oral	2/week	3 weeks	11 ± 1·0
Vitamin A + calciferol		Vit. A—20 I.U.	I.P.	daily	2 weeks	17 ± 2·0
		Calciferol—2 I.U.	oral			
		,,	,,	daily	4 weeks	16 ± 8·0
Aneurin	0·02 mgm.	0·06 mgm.	oral	daily	1 week	17 ± 2·1
Pyridoxine	0·02 mgm.	0·08 mgm	oral	daily	1 week	19 ± 3·1
Nicotinic acid	0·1 mgm.	0·2 mgm.	oral	daily	1 week	14 ± 1·9
Inositol	?	40·0 mgm.	oral	daily	1 week	18 ± 1·5
Biotin	0·0008 mgm.	0·006 mgm.	oral	daily	1 week	14 ± 0·6
Pantothenol	0·2 mgm.	1·0 mgm.	oral	daily	1 week	15 ± 0·5
Choline	4·0 mgm.	40·0 mgm.	oral	daily	1 week	20 ± 1·8
Cyanocobalamine	0·00008 mgm.	0·0016 mgm.	I.P.	daily	1 week	17 ± 1·2
Ascorbic acid	?	1·0 mgm.	oral	daily	1 week	15 ± 1·2
α-Tocopherol	0·1 mgm.	2·0 mgm.	oral	daily	1 week	16 ± 1·3
Methylnaphthyline di(dihydrogen) phosphate	0·02 mgm.	0·4 mgm.	oral	daily	1 week	15 ± 1·6
Naphthaquinone	0·02 mgm.	0·4 mgm.	oral	daily	1 week	15 ± 1·0
Aneurine Riboflavine Pyridoxine Nicotinic acid Ascorbic acid		0·4 mgm. 0·8 mgm. 0·4 mgm. 2·0 mgm. 1·0 mgm.	oral	daily	1 week	17 ± 1·7
Controls						15 ± 2·0

of administration was either intraperitoneal or by stomach tube.

Two days after completion of the vitamin treatment the phagocytic activity was measured in each group of animals by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[4], the procedure used being that described in a previous communication[3], and the total body phagocytic activity or phagocytic index being denoted by the symbol K.

Ten control animals, receiving no treatment, showed a phagocytic index or K value of $15 \pm 2{\cdot}0$.

The results are shown in Table 1. In the doses administered the vitamins given to normal healthy growing mice had no discernible effect on the phagocytic activity of the reticulo-endothelial system. The above results might well have been expected since, for the most part, vitamin action appears to be related to the enzyme systems of normal cellular metabolism. In contrast, in infection, when the metabolism of the reticulo-endothelial cells increases, the requirement for the various vitamins may also be raised.

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Effect of Bradykinin on Uterine Activity

It was reported recently[1] that plasma kinin is formed spontaneously in human plasma collected during labour. Tested on the isolated rat uterus, the maximum oxytocic activity was stated to be equivalent to several units of oxytocin per millilitre of plasma. It was suggested that kinin formation may be related to the powerful contractions of the human uterus during parturition.

As yet the only kinin which has been chemically defined and is available in a pure form is bradykinin. We therefore investigated the uterine effect of synthetic bradykinin[2] on the human uterus *in situ* (external[3,4] and internal[5] tocography) and *in vitro*[6], and on the uterus *in situ* of anæsthetized non-pregnant rats, cats[7] and rabbits[8].

Single intravenous injections of up to 2 μgm./kgm. bradykinin and intravenous infusions of 0·14, 0·28 or 0·56 μgm./kgm./min. of bradykinin—each dose infused for a period of 5 min.—had no oxytocic effect on the human uterus *in situ* a few hours before or after labour. In one case, 1·4 μgm./kgm./min. infused for 3 min. was followed by a contraction, which may have been connected with the administration of the peptide. Although no uterine effect could be detected, the administration of higher doses of bradykinin elicited a number of symptoms, such as lowering of the systemic blood pressure, hot flushes, sensation of warmth in various parts of the body, etc.

Isolated strips of human myometrium obtained at Cæsarian section contracted only after bradykinin doses as high as several hundred or even thousand μgm./l. Some strips failed to respond even to 10,000 μgm./l., although their sensitivity to oxytocin ('Syntocinon^R'), for example, 25–100 milli-units/l. was normal (100 milli-units oxytocin is equal to 0·2 μgm. peptide).

The rat uterus *in situ* usually gave a short-lived contraction with 100 μgm./kgm. bradykinin (intravenous); 10 μgm./kgm. elicited a response only in a few animals. 100 milli-units/kgm. oxytocin consistently produced a response in all the rats.

In the cat uterus *in situ* 100 μgm./kgm. bradykinin (intravenous) invariably elicited a short-lived response. In many animals 33 μgm./kgm. or 10 μgm./kgm. was also effective, and one responded to as little as 3·3 μgm./kgm. All the cats displayed a characteristic uterine reaction to 40–80 milli-units/kgm. of oxytocin.

The sensitivity of the rabbit uterus *in situ* to bradykinin varied greatly. Whereas in some rabbits the intravenous injection of 1 or 10 μgm./kgm. was followed occasionally by a brief contraction of the uterus, others did not react even to 100 μgm./kgm. (intravenous). All rabbits showed a normal sensitivity to methyl-ergometrine ('Methergin^R'): 0·15 mgm./kgm. invariably elicited the usual uterotonic reaction.

The isolated rat uterus is known to have a very high sensitivity to bradykinin: a minute dose of 0·00003 μgm./ml. will sometimes elicit a contraction[9]. However, the findings presented in this communication show that bradykinin can scarcely be regarded as oxytocic in the usual sense of the word. The doses of bradykinin required to elicit a contraction of the rat, cat and rabbit uterus *in situ* are usually much higher than those evoking a marked fall of blood pressure. Moreover, the contraction is of a shape different from that produced by a low dose of oxytocin. The amounts of bradykinin which contract the isolated human myometrium are extremely high, and on the human uterus *in situ* bradykinin is virtually devoid of any effect, even in doses producing considerable distress.

These findings do not favour the view that bradykinin might be a physiological oxytocic factor responsible for increased uterine activity during labour in the human being.

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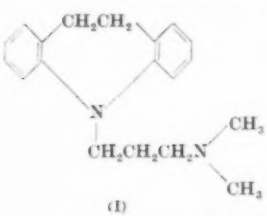
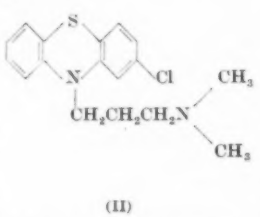
XUM

Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in Experimental Animals

Imipramine (I) is a drug that is related chemically to the major tranquillizers of the phenothiazine group such as chlorpromazine (II).



(I)



(II)

As distinct from chlorpromazine, however, imipramine is claimed to be of considerable value in the treatment of depression, particularly of the endogenous type[1–3]. Pharmacologically, imipramine has many of the properties of chlorpromazine although it is quantitatively less potent. Thus in the mouse, imipramine, like chlorpromazine, reduces motor activity, prolongs hexobarbitone sleeping time, and causes a fall in rectal temperature[4,5]. Both imipramine and suitable doses of chlorpromazine counteract the hypothermia, ptosis, bradycardia, and diarrhœa caused by reserpine administration in rats[6], and both drugs produce sedation in the cat and dog[4]. In the curarized cat, both drugs block the electrocortical desynchronization caused by electrical stimulation of the mesencephalic reticular formation[4], although it has been suggested[7] that this action of imipramine is due to atropine-like properties rather than to chlorpromazine-like sedation. Slight qualitative differences have been observed between chlorpromazine and imipramine in their action on the electrical activity recorded from certain sub-cortical areas in the rabbit. The resemblance between the pharmacological actions of these two drugs has led to the view[8] that the differences between the pharmacological effects of chlorpromazine and imipramine are, at present, only quantitative, rather than qualitative. Furthermore unlike other drugs, such as iproniazid and nialamide, used at present in the treatment of depressive illnesses, imipramine is not an inhibitor of mono-amine oxidase[9]. There appeared, therefore, to be no pharmacological basis for its important clinical anti-depressant properties.

We wish to report on the action of imipramine in two laboratory tests which clearly reveal actions indicative of central stimulant or anti-depressant properties and which are qualitatively different from those of chlorpromazine and related phenothiazine derivatives.

(*a*) *Production of secondary conditioned responses in the rat.* A conditioned avoidance situation similar to that previously described[10] was used, in which rats were trained to climb a vertical rope at the sounding of a buzzer (conditioned stimulus), in order to avoid an electric shock (unconditioned stimulus). The three types of response which conditioned rats may show when placed in this situation are: (1) climbing the rope at the first conditioned stimulus (this is the response shown by most fully conditioned animals); (2) climbing the rope after a number of conditioned stimuli, or not climbing at all (this indicates that the conditioned reflex has been partly or completely inhibited); (3) climbing the rope before the conditioned stimulus is given. This last type of response has been termed a secondary conditioned response[11]. The ability of imipramine and other drugs either to block the conditioned reflex or to induce a secondary conditioned response was determined at intervals after administration. All the rats used in this investigation were selected from those giving consistent performances and climbing on the first conditioned stimulus.

(*b*) *Reversal of reserpine-induced ptosis in the rabbit.* In order to demonstrate an anti-reserpine property of imipramine we used the following method: Reserpine was infused intravenously into conscious rabbits restrained in a stock until a dose of 2·5 mgm./kgm. had been administered. Approximately 30 min. after the

Table 1. Activities of Imipramine, Chlorpromazine, Dexamphetamine and Cocaine in producing Secondary Conditioned Responses in the Rat and in reversing the Action of Reserpine in the Rabbit

Substance	Route	Conditioned reflex test (rat)			Behavioural changes in the rat	Reserpine reversal. Effective dose intravenous (rabbit) (mgm./kgm.)
		Conditioned reflex	Secondary conditioned response			
		Effective dose for block of conditioned reflex	Dose (mgm./kgm.)	Per cent development of secondary conditioned response		
Dexamphetamine sulphate	s.c.	>4·0	0·25 0·5 1·0 2·0 4·0	20 50 80 60 40	General stimulation, exophthalmos, marked increase in motor activity, jerky head movements, fighting between rats	1·5±0·3
Cocaine hydrochloride	s.c.	>10·0	0·6 2·5 10·0	30 50 70	General stimulation, exophthalmos, no increase in motor activity. Rats show increase in alertness and reactivity. Jerky head movements, some disorientation.	6·8±0·6
Imipramine hydrochloride	p.o.	>40·0	5·0 10·0 20·0 40·0	20 40 25 10	Exophthalmos up to first hr. after dosing. Appearance becoming normal or showing slight ptosis. Initial increase in motor activity. No sedation. Most rats are sedated. Complete or partial ptosis, reduction in motor activity. Resemble chlorpromazine-treated animals.	15±1
Chlorpromazine hydrochloride	s.c.	3·0	—	—	General sedation, complete or partial ptosis, reduction in motor activity, some loss of muscle tone.	>40

reserpine infusion, the rabbits developed symptoms of sedation including loss of reactivity, ptosis, hypothermia, and often loss of the righting reflex. This central depression was marked 1 hr. after the reserpine, and lasted for at least 4 hr. One hour after the administration of reserpine, a dilute solution of the drug under test was infused intravenously at the rate of 0·4 ml./min. The quantity of drug required to reverse the reserpine sedation completely was measured. We found that the degree of ptosis of the animals was the most satisfactory criterion of reserpine-induced sedation, since this followed closely the level of general reactivity and enabled a sharp end-point of the reserpine reversal to be obtained.

Table 1 sets out the activities of imipramine in these two tests in comparison with chlorpromazine, dexamphetamine and cocaine. Inhibitors of mono-amine oxidase, such as iproniazid and nialamide, were inactive in the two procedures.

At low doses in the rat, imipramine showed clear-cut stimulant properties; but as the dose was increased, chlorpromazine-like central depressant properties became more apparent. In the rabbit, complete reversal of reserpine-induced ptosis was observed after intravenous infusions, and if the infusion were continued exophthalmos became prominent. These changes were accompanied by an increase in alertness and general reactivity.

The fact that iproniazid and nialamide are inactive in these tests whereas imipramine shows clear-cut activity suggests that the mechanism of the anti-depressant action of these drugs is quite different. Sigg[4] has suggested, from experiments on the nictitating membrane of the anæsthetized cat, that imipramine bears some resemblance to cocaine in its peripheral actions and that its anti-depressant effect may be due to sensitization of central adrenergic synapses. Although we have no evidence on this point, imipramine would appear to differ from cocaine in that it shows stimulant properties at low doses only, while at high doses a chlorpromazine-like type of sedation is observed.

It is of interest that the effective doses of dexamphetamine, cocaine and imipramine in the tests in the rat and rabbit are of the same order of magnitude and this suggests that the same central property is being measured in the two procedures. Whether this property is the one which in man is responsible for the clinical anti-depressant effects we cannot say. However, these experiments clearly differentiate imipramine from chlorpromazine and show its resemblance to the classical central stimulants.

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Role of the Lung in Metabolism of Fat during Ontogeny of the Rat

Milk of the rat contains up to 20 cer cent fat[1], and the gastro-intestinal tract of infant rats absorbs fat more rapidly than that of adult animals[2]. The cells of the intestinal mucosa of infant rats after a meal contain much more neutral fat than those of adult animals where more 'acid lipids' are found[3] (using nile blue). This, together with a lower intestinal esterase activity in infant rat intestine[2], indicates a different absorption and perhaps transport of fat. Since fat transported via lymphatic pathways must pass through the lungs before entering the blood, it was thought pertinent to examine this organ histochemically during ontogeny of the rat.

First, infant rats (1 and 10 days post-natally) taken directly from the mother animal with their stomachs full of milk were compared with animals aged 45 days (post-weaning stage) and adult rats. As is evident from Fig. 1, fat accumulates in the adventitia of the arterioles and arteries and in the walls of the alveoli in infant rats aged 1 and 10 days. No fat is found in the macrophages. In animals aged 45 days and adult rats there is no fat. Only occasionally, macrophages are seen having fat droplets in their cytoplasm. Complete starvation for 24 hr. resulted in disappearance of all fat from the lungs of infant rats. In adult animals no changes were observed.

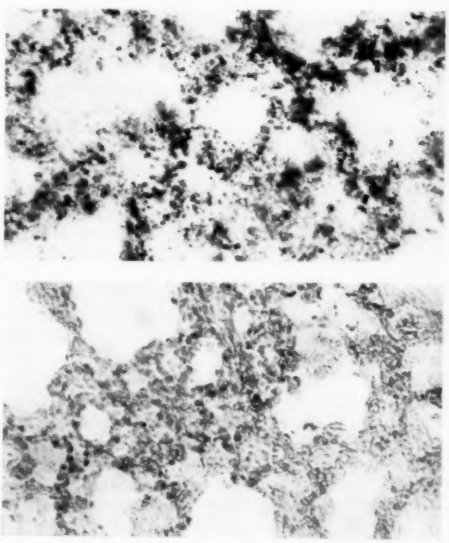

Fig. 1. (*a*) Lung of 10-day-old rat after feeding with milk. Frozen section, oil red 7*B* (× 90). (*b*) Lung of adult rat after feeding. Frozen section, oil red 7*B* (× 90)

In further experiments olive oil or oleic acid (1,000 mgm./100 gm. body-weight) was administered to starving rats and the animals were killed 1 hr. and 3 hr. and, for adult rats, also 7 hr. later. The histological picture was exactly the same as in fed animals. Again fat was found in the lungs of suckling rats and none in adult animals.

Schrade and Biegler[4] also found no fat histologically in the lungs of adult rats following a fatty meal. On the other hand, they found an increase in fat content of the lungs as determined chemically. This might indicate that the fat in the lungs is present in a

different form in adult and infant rats, and that this might be due to a difference in transport of fat and in the part played by the lungs in this process. A preliminary experiment using a 5-day-old puppy showed the same picture as in infant rats.

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Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission

The nerve supply to many sympathetic effector organs contains cholinergic nerve fibres as well as adrenergic fibres. Recently, the hypothesis has been put forward that a cholinergic mechanism is involved in sympathetic transmission[1,2]. It has been suggested that acetylcholine released from cholinergic nerve endings liberates from a local tissue store sympathetic transmitter which in turn acts on the effector organ.

One of the ways in which this hypothesis has been tested has been to use a hemicholinium, HC3 (ref. 3), which inhibits the formation of acetylcholine[4,5]. If sympathetic transmission does depend on the release of acetylcholine the effect of stimulating post-ganglionic sympathetic nerves should be blocked by HC3. Rand and Chang[6] showed that HC3 blocked the contractions of the isolated ductus deferens of the guinea pig produced by stimulation of the sympathetic nerve. Later they showed similar effects on a number of other sympathetic effector organs, including the isolated uterus and colon of the rabbit, the perfused rabbit ear, the isolated atria of the cat and the piloerector muscles of the cat's tail, most of which are known to contain both cholinergic and adrenergic fibres in their nerve supply[7].

To determine whether adrenergic transmission is necessarily mediated via a cholinergic mechanism we have used the nictitating membrane of the cat. This can be set up as an isolated nerve–smooth muscle preparation[8], and the evidence is that under these conditions neuromuscular transmission is purely adrenergic[8]. The post-ganglionic nerve fibres were stimulated with supramaximal square-wave shocks of 0·5 msec. duration at frequencies of 5, 10, 20, 25, 50 and 100 c./s. for periods of 7 sec. repeated every minute. HC3 in concentrations of 20–540 μgm./ml. ($=3{\cdot}5 \times 10^{-5} - 9{\cdot}4 \times 10^{-4}\ M$) had no effect on the contractions of the muscle in response to nerve stimulation; even when stimulation was carried out in this way for more than 1 hr., there was no effect. These findings confirm those reported by Wilson and Long[9], who tested the action of HC3 on the nictitating membrane *in vivo*.

HC3 prevents the formation of acetylcholine not by inhibiting choline acetylase directly but by preventing the passage of choline through cellular membranes which enclose the enzyme[5]. The blocking effect of HC3 may not therefore become apparent unless the duration and frequency of nerve stimulation are sufficient to deplete the stores of acetylcholine[4]. In our experiments the higher frequencies of intermittent stimulation were greater than those which were required to produce an effect on the test objects used by Chang and Rand[7]. Nevertheless, the possibility remained that HC3 would not block the contractions of the nictitating membrane unless the nerve were stimulated continuously. When this was done at frequencies of 25 c./s. and 100 c./s. the contraction produced began to wane almost immediately, but the presence of HC3 in concentrations of 60–100 μgm./ml. ($=1{\cdot}05 \times 10^{-4}$–$1{\cdot}75 \times 10^{-4}\ M$) did not alter the rate at which the contraction decayed, even when HC3 was present for as long as 40 min.

Another indication that transmission is cholinergic might be that choline prevents or reverses the waning contraction produced by continuous stimulation at high frequencies. It was found, however, that choline in concentrations of 1·4–200 μgm./ml. ($=10^{-5}$–$1{\cdot}42 \times 10^{-3}\ M$) did not diminish the rate of decay.

These results do not support the suggestion that a cholinergic mechanism is concerned in sympathetic transmission over adrenergic nerve fibres.

We wish to thank Dr. M. J. Rand and Dr. H. Wilson for generous supplies of HC3. Experiments in which these gifts of HC3 were used gave results identical to those obtained with HC3 prepared by one of us (J. E. G.).

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HISTOCHEMISTRY

Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat

Morphologically the mammary gland is a cutaneous gland, but physiologically it must be classed as an accessory gland of the reproductive system. In the various stages of the life of the female mammals the mammary gland undergoes many structural changes. Up to the age of puberty it is rudimentary in structure. During pregnancy the endocrine factors furnish a stimulus for proliferation of the mammary tissue, resulting in a complete transformation and maturation of the gland. The appearance of fine granulations and fat droplets in the epithelium cells indicates the onset of secretion in advanced pregnancy.

To our knowledge, the activity of the histochemically demonstrable leucine aminopeptidase in the mammary tissue has not been studied previously; therefore the object of our present work was to investigate the leucine aminopeptidase activity in the mammary gland of the cow and rat in the various stages of development.

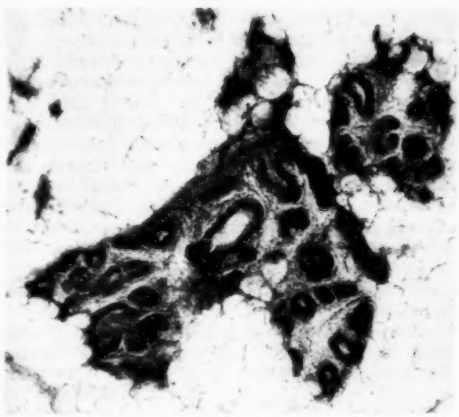

Fig. 1. Great activity of leucine aminopeptidase in the mammary tissue of a pregnant rat. (× 50)

The method employed in the demonstration of the leucine aminopeptidase activity in the tissue was the original method of Nachlas *et al.*[1]. The mammary glands were taken from virgin, pregnant and lactating animals. The parts of glands were cooled with dry ice and sectioned with the Pearse cold microtome[2]. The incubation time was 15–30 min.

The positive areas were characterized by a violet stain. In the mammary glands from virgin and pregnant animals a strong activity of leucine aminopeptidase was observed. This activity was located in the walls of the glandular ducts and also in the epithelium buds representing the glandular tissue in the non-functional gland. These buds were mainly without lumina. An example of this activity is shown in Fig. 1. The connective tissue including adipose tissue was nearly negative. In the lactating gland the activity was very slight (Fig. 2). Its location was the same as that in the non-functional gland. Especially in the alveolar epithelium the activity was minimal, in fact practically absent.

From the physiological point of view, the result is difficult to explain. The role of leucine aminopeptidase in tissues is still obscure. Observations made by Pearse and Tremblay[3] indicate that leucine aminopeptidase participates in the protein synthesis of the parathyroid glands. It also seems likely that the leucine aminopeptidase of the cells of the gastric mucosa is responsible for the pepsin synthesis[4]. This enzyme is, however, not associated with the casein synthesis of the mammary gland during lactation according to the present results. Apparently, the protein synthesis may be independent of the activity of the histochemically demonstrable leucine aminopeptidase.

Further investigations on the effect of various hormones on the leucine aminopeptidase in the mammary tissue are in progress.

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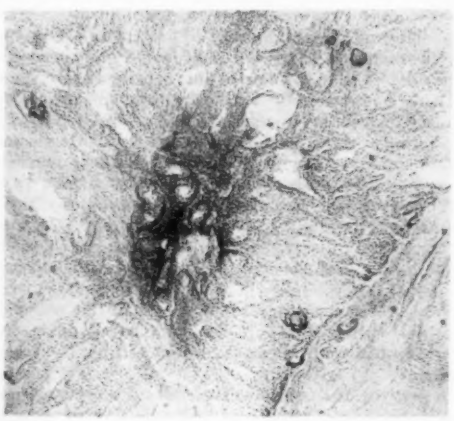

Fig. 2. Mammary gland of a lactating rat. Activity very slight. (× 50)

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PATHOLOGY

Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a Relatively Avirulent Strain of *Salmonella typhimurium*

Although previous specific active or passive immunization may prolong slightly the survival-time of mice infected with mouse-pathogenic *Salmonellæ* (for example, *Salm. typhimurium*, *Salm. enteritidis*), the number of long-term survivors is but little improved[1–3]. Similarly, attempts to enhance resistance against mouse typhoid non-specifically, either by previous infection with *Mycobacterium tuberculosis* (strain *BCG*)[4] or by injections of lipid *A* from Gram-negative bacteria[5], have also met with only limited success. On the other hand, Hobson[3] showed that mice were highly resistant to virulent *Salm. typhimurium*, in terms of survival, if they had been infected previously with an avirulent variant of the same species. As *Salm. typhimurium* is a reticulo-endothelial parasite in mice, like *Myco. tuberculosis* and *Brucella abortus*, the possibility arises that this greatly increased resistance might resemble the cellular antibacterial immunity mechanism inferred from investigations with these latter micro-organisms[6–8]. Evidence has been obtained, however, both from *in vitro* macrophage studies and mouse infection experiments, which suggests that 'cellular immunity' following infection with *Myco. tuberculosis* cross-reacts against *Brucellæ* and vice versa[9–12]. The present communication illustrates that there is also a considerable non-specific element in the resistance produced by avirulent *Salm. typhimurium*, for this has been found to be effective against a heterologous *Salmonella* infection. *Salm. enteritidis* was considered unsuitable for use in this role as it shares somatic antigen components with *Salm. typhimurium*. Instead, *Salm. paratyphi C* (6,7,*Vi* : *c* : 1,5) was selected as it shares only one second-phase flagellar antigen with *Salm. typhimurium* (1,4,5,12 : *i* : 1,2) and,

although not a natural mouse pathogen, produces an experimental infection similar to mouse typhoid.

A group of 30 female mice of the *LAB* Grey outbred stock (weight 20–23 gm.) was infected with *Salm. typhimurium* (strain *S2/R*) by injecting intravenously 1,000 bacteria taken from a 6-hr. broth culture. This was the same relatively avirulent strain of *Salm. typhimurium* previously used by Dr. Derek Hobson[2] and kindly provided by him. After 13 days, at which time no deaths had occurred, 5 animals were killed and viable bacterial counts made on the homogenized liver and spleen of each. *Salm. typhimurium* was recovered in all cases with counts varying between 10,000 and 50,000. On the following day (day 14), the phagocytic activity of the reticulo-endothelial system was investigated in a further 5 mice, by following the rate of disappearance of a standard dose of colloidal carbon from the circulating blood according to the method of Biozzi and his colleagues[13]. The results in Table 1 show that the reticulo-endothelial system was more than twice as active, when assessed by the phagocytic index K, than in a group of control mice. Furthermore, the mice parasitized with *Salm. typhimurium* showed some relative enlargement of the liver and spleen, although this was pronounced in only 2 out of the 5 animals examined. The remaining 20 mice treated, together with 20 normal animals, were then injected intravenously with *Salm. paratyphi C* (Kauffmann East Africa strain) at a dose of 100,000 bacteria from a 6-hr. broth culture. Whereas 100 per cent mortality occurred in the control group, only one mouse died among those pre-infected with *Salm. typhimurium* during a 30-day period of observation (Table 2). *Salm. paratyphi C* was isolated from the heart blood of this sole fatality.

It seems improbable that such a high degree of resistance, which compares favourably with the result of active immunization against *Salm. paratyphi C* in mice[14], could be explained by the minor flagellar antigenic relationship between these *Salmonella* species (unless, of course, *H* antigens assume an importance in this resistance following parasitization which they do not possess in orthodox immunization procedures). Neither would the raised phagocytic activity of the reticulo-endothelial system alone provide a satisfactory explanation, for greater degrees of stimulation induced by other means have failed to protect against infections of this type[15,16]. In some way, previous parasitization of the reticulo-endothelial cells by *Salm. typhimurium* would seem to inhibit subsequent multiplication within those cells of a secondarily invading *Salmonella*[3], even when this does not necessarily belong to the same or a closely related species.

This investigation was carried out at the Wright-Fleming Institute, St. Mary's Hospital Medical School, London.

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Table 1. Activity of the Reticulo-Endothelial System in Mice infected with *Salm. typhimurium* (strain *S2/R*, 1,000 Bacteria Intravenously) 14 days Previously. The Clearance of 16 mgm./100 gm. Colloidal Carbon (C11/1431*a*) was measured

Group	Phagocytic index K*	$\frac{W}{WLS}$†
Mice infected with *Salm. typhimurium* 14 days previously	0·036	15·7
	0·041	13·5
	0·043	13·7
	0·056	8·5
	0·064	8·9
Mean	0·048	12·5
Controls	0·017	23·2
	0·018	16·5
	0·025	16·1
Mean	0·020	18·6

* $K = \frac{\log c_1 - \log c_2}{t_2 - t_1}$, where c_1 and c_2 are the concentrations of carbon in the blood at times t_1 and t_2 (min.)
† W = body-weight (gm.)
WLS = weight of liver and spleen (gm.)

Table 2. The Results of Infection with *Salm. paratyphi C* (100,000 Bacteria Intravenously) in Normal Mice and Mice infected with Avirulent *Salm. typhimurium* (Strain *S2/R*, 1,000 Bacteria Intravenously) 14 days Previously

Group	Mice dead / Mice injected	Time to death (days)
Mice infected with *Salm. typhimurium* (*S2/R*) 14 days previously	$\frac{1}{20}$	8
Controls	$\frac{20}{20}$	5,5,5,6,6,6,6,6,6,6, 6,7,7,7,7,7,7,9,11,11

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ANATOMY

Functions of the Mammalian Carotid Rete Mirabile

Although a carotid rete mirabile is present in many mammals, notably the artiodactyla, its function is uncertain. Many workers have suggested that the rete may regulate the cerebral blood-flow[1,2], but emphasis has usually been laid on its effect on the arteries rather than the veins. However, an alternative possibility is that the rete is concerned primarily with assisting the venous return from the interior of the cranium, which is especially liable to be embarrassed in animals that feed with the head dependent. Enhancement of the blood-flow in veins is known to be a function of certain retia mirabilia elsewhere[3].

When present, the carotid rete always lies within a venous lake, usually the cavernous sinus[1]. In man, the expansile pulsations of the internal carotid artery are said to assist the expulsion of venous blood from the sinus[4], but a single thick-walled artery has limited powers of expansion[5]. Replacement of one artery by multiple thin-walled channels must enhance considerably the arterial expansion and, thus, the venous return.

In general, the evidence for such a pumping mechanism is the demonstration of pulsatile flow in the veins draining a part, coincident with the pulsations in the arteries of supply. In the umbilical vein, for example,

such coincident pulsations have been recorded[6]; these are certainly due to direct pressure from the companion arteries in the umbilical cord. We have obtained comparable results with respect to the carotid rete of the sheep. In two heads from freshly slaughtered sheep the jugular vein and the common carotid artery were cannulated in the neck, and warm water was perfused via the artery at a pressure of 100 mm. of mercury. The venous pressure settled at 33 cm. of water. Rhythmic occlusion and release of the inflow channel gave rise to coincident pulsations in the venous flow, and the venous pressure rose by 12 cm. and overflowed the manometer tube. In a third sheep's head the vein cannulated was a large one, draining the extracranial tissues only. Rhythmic arterial pulsations had no effect on the flow in this vein, an indication that the venous pulsations noted in the first two experiments were directly transmitted from the arterial vessels to the venous lake within the cavernous sinus, the rete mirabile acting as a venous pump.

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BIOLOGY

Sex-Ratio in Mice

The impression gained from glancing through our mouse record cards suggested to us an unusual sex-ratio in some of our strains; some strains appeared to be consistently producing more males than females or vice versa. As impressions are notoriously misleading, it was decided to carry out a brief analysis.

Mice are produced and recorded at the Laboratory Animals Centre, Carshalton, in the manner described by Bartlett *et al.*[1]; under our system mice are sexed not at birth but at weaning. Because of this, the only litters included in the sex analysis were those where all the mice survived until nineteen days of age (that is, until weaning). Total numbers of litters per strain were considered to ascertain whether the proportion analysed could be correlated with abnormal sex-ratio. The period under review was March 1955, until February 1959.

Results obtained from this analysis are recorded in Table 1. Columns 2, 3 and 4 give the figures necessary for analysis of the litters, and columns 5, 6 and 7 those necessary for the sex analysis of the mice from litters where there was 100 per cent survival. The expected numbers of each sex for each strain were calculated on the basis that the male : female ratio equals 1 : 1 and a χ^2 goodness-of-fit test applied to observed and expected numbers of each sex. χ^2 values and probability-levels (P) are tabulated for each strain.

The results show that in the case of the *A*, *C57BR/cd* and the *LAC* albino strains, significantly more females than males were produced whereas the *CE* strain produced more males than females. Other strains showed a more normal sex ratio. No correlation is evident between survival of the whole litter and sex ratio.

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Methæmoglobin in Chronic Copper Poisoning of Sheep

After protracted periods of high copper-intake some species of farm animals accumulate large excesses of copper in the liver, and in certain circumstances this copper can be suddenly released into the blood and the clinical entity known as chronic copper poisoning develops[1–4]. Sheep are particularly susceptible, and the sequence of clinical symptoms is generally: anorexia, hæmolysis of red cells, hæmoglobinuria, anæmia, jaundice and death. Although the build-up of copper in the liver may take place over a period of weeks or months, the 'hæmolytic crisis' is an acute illness and death usually occurs within 2–3 days.

Although hæmolysis and hæmoglobinuria are constant features[5,6], the occurrence of methæmoglobin does not appear to have been recorded. In outbreaks of chronic copper poisoning[3,7], it has been observed that blood samples from affected animals are often chocolate brown in colour, and spectroscopic

Table 1

Strain	1 No. of pairs	2 No. of litters Total	3 No. of litters 100 per cent survival	4 Per cent	5 Total No. of mice	6 No. of ♂	7 No. of ♀	χ^2	P
A	57	333	184	55	775	345	430	9·32	0·01–0·001
C57BR/cd	65	346	242	70	1,413	638	775	12 90	0·01–0·001
LAC Albino*†	104	515	316	61·3	2,170	1,028	1,142	5·988	~ 0·02
CE	57	232	178	76·7	1,369	730	639	6·048	0·01–0·02
Balb/*C*	87	499	388	77·8	2,029	999	1,030	0·04736	0·95–0·05
C3H/He	67	378	211	55·8	1,176	591	585	0·00306	0·95–0·05
LAC Grey*	144	819	689	84·1	5,397	2,765	2,632	3·328	0·95–0·05
DBA/1	49	247	161	65·1	665	318	347	3·3218	0·95–0·05
A2G	70	401	284	70·8	1,528	734	794	2·356	0·95–0·05
CBA	46	223	126	56·5	796	397	399	0·005024	0·95–0·05
C57BL	45	155	94	60·6	445	231	214	0·6494	0·95–0·05

* Non-inbred strains. † Strain discontinued 1959.

examination showed the presence of methæmoglobin. Estimations of methæmoglobin and total hæmoglobin by the method of Evelyn and Malloy[8] have been carried out on a number of cases and typical results from three affected sheep are set out in Table 1, with normal levels for comparison.

Table 1. Total Hæmoglobins, Methæmoglobin and Copper-Levels in Whole Blood in Chronic Copper Poisoning

	Blood copper (μgm./100 ml.)	Total hæmoglobins (gm./100 ml.)	Methæmoglobin (gm./100 ml.)
Case 1	1,210	11·5	3·5
Case 2	1,480	8·8	3·6
Case 3	1,000	15·5	3·8
Normal sheep	170	10 to 12	0·1

Copper-levels in blood were extremely high and methæmoglobin constituted 25 per cent to 35 per cent of the total hæmoglobin present. The methæmoglobinæmia was mainly intracorpuscular, but since hæmolysis was taking place, hæmoglobin and methæmoglobin were being released into the plasma, the proportions being approximately the same as occurring in whole blood, for example, plasma total hæmoglobins 2·2 gm. per 100 ml.; methæmoglobin 0·8 gm. per 100 ml. (36 per cent).

The pathological significance of these findings is being investigated.

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Release of Heparin from the Mast Cells of the Rat

Mast cells are an important source of tissue heparin and of histamine[1,2]. Histamine is known to be released following disruption of mast cells[3] and in the dog there is an associated release of heparin[4]. In other animals it has not been possible to detect liberation of heparin coincident with release of histamine.

The liberation of heparin from rat mast cells has been investigated *in vitro* following disruption of the mast cells. Cells were obtained from the peritoneal cavity of the rat by a method already described[3]. Mast cells were separated from other peritoneal cavity cells by centrifugation for 30 min. at 0° C. over a column of bovine albumin of 1·109 specific gravity. The mast cells passed through the albumin column and were collected at the bottom of the centrifuge tube. They were washed in physiological saline solution and suspended in saline solution in a concentration of approximately 0·5 million mast cells per ml. of solution. The mast cells in 1 ml. of suspension were disrupted by the following methods: (1) alternately freezing and thawing three times; (2) mechanical disruption in a piston and cylinder type homogenizer; (3) addition of compound 48/80 to give a final concentration of 2×10^{-6} mgm./ml.; (4) osmotic disruption with distilled water; (5) disruption by contact with an antigen–antibody mixture as already described[3]. This mixture consisted of 1 vol. of a 25 per cent suspension of human red cells in physiological saline solution and 5 vol. fresh rat antiserum at 37° C. and *p*H 7. After disruption of the mast cells, the preparations were centrifuged and the supernatant solutions assayed for antithrombin activity by the method of Monkhouse and Jaques[5].

The only supernatant solution of the disrupted mast cells found to have any antithrombin activity was the preparation that had been frozen and thawed three times. Activity equivalent to approximately 3·5 I.U. of heparin was released into the supernatant solution for each million mast cells frozen and thawed. Antithrombin activity remained uniform throughout the supernatant after centrifugation for 1 hr. at 28,000*g* in a 'Spinco' ultra-centrifuge. A residual small amount of activity, less than the equivalent of 0·5 I.U. heparin per million mast cells, was found in the deposit of the disrupted cells.

If the deposits of the other four solutions were digested with trypsin at *p*H 8·0 and then neutralized, the solutions showed antithrombin activity equivalent to approximately 4 I.U. heparin per million mast cells. This value agreed with the antithrombin content of untreated mast cells. As with heparin, the activity was readily neutralized by small amounts of protamine sulphate.

Microscope examination of stained smears of the disrupted mast cells revealed disruption of the granules only in the preparations which had been frozen and thawed.

It may be concluded that the antithrombin present in rat mast cells is not released into the surrounding solution following disruption of these cells *in vitro* unless the granules also are disrupted. This is in contrast to the results with histamine, which is known to be released from disrupted rat mast cells even though the granules remain intact.

Compound 40/80 used in these experiments was kindly made available by Burroughs Wellcome and Company (Aust.), Ltd.

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Mast Cells in Photosensitivity Reactions

In recent work[1] it was found that cells obtained by washing out the rat's peritoneal cavity with saline could be rendered photosensitive. Exposure to light for a period too brief to affect leucocytes and serosal cells caused the granules of sensitized mast cells to lose their basophilic staining properties, after which they became refractory to the action of the histamine-releaser, compound 48/80. Mast cells reacted in this way even after being separated from the other cells by differential centrifugation.

In order to determine whether histamine had actually been liberated when mast cells lost their

basophilic staining reaction, the cells from six rats were obtained by washing out their peritoneal cavities by the method I have described[2]. The washings were centrifuged and the deposit was re-suspended in the fluid which ran down the sides of the tubes. The cell suspension was then divided into three parts.

To two groups hæmatoporphyrin was added to an eventual concentration of 1 part in 20,000 and to the third group was added an equivalent volume of physiological saline without hæmatoporphyrin. One preparation containing hæmatoporphyrin was kept in the dark while the other two were exposed to bright sunlight for 2 min. in watch glasses. The suspending fluid was separated from each by centrifugation and the cells were re-suspended in saline containing compound 48/80 to a concentration of 1 part in 20,000.

After 2 min. the preparations were again centrifuged. The two supernatants from each and the cellular deposits were then all tested for histamine according to the method of Lowry *et al.*[3]. It was found that hæmatoporphyrin did not cause release of histamine in either the preparation exposed to light or in the one kept in the dark. Nor was there any release from the preparation exposed to light without hæmatoporphyrin. The addition of compound 48/80 caused the release of histamine from cells of the two control preparations, but in the case of the cells which had been exposed to light in the hæmatoporphyrin solution, all the histamine remained within the cells. Addition of a drop of 0·5 per cent aqueous azur II solution to a drop of cell suspension confirmed the loss of basophilia by mast cells exposed to light in the presence of hæmatoporphyrin.

When exposure to light of the sensitized cells had not been adequate for complete abolition of basophilia, any granules which retained their staining power were extruded on the addition of compound 48/80.

These features seem to indicate that release of histamine from mast cells depends in some way on the presence of basophilia. The loss of basophilia, however, does not indicate death of mast cells. Exposure to a constant source of light from a slide projector showed that 1 part of hæmatoporphyrin in 20,000 removed mast cell basophilia within 1 min., whereas death of mast cells, which was recognizable by staining of their nuclei by the non-vital stain azur II, occurred only after exposure for 15 min.

Eosin and erythrosin were tested against the rat's peritoneal cells and they, too, caused mast cells to yield their basophilic staining properties when exposed to light, and, as usual, the cells thereby became refractory to compound 48/80.

Apart from these substances, which are active only on exposure to light, there are other substances such as chlorpromazine and promethazine which cause loss of mast cell basophilia in the dark with enhancement of this property on exposure to light. Each of these is a histamine-releaser causing swelling and disruption of mast cells in dilutions up to approximately 1 part in 20,000 parts of saline. In these concentrations they do not affect the basophilia of mast cells, but in dilutions between 1 : 20,000 and 1 : 40,000, they cause mast cells to lose their basophilic staining properties and on exposure to bright sunlight for 2 min. the range is extended to 1 : 80,000, even to 1 : 160,000 in occasional animals.

In addition to the reactions of photosensitivity, heating to 50° C. for 40 sec. causes mast cells to lose their basophilia and become refractory to the action of compound 48/80. Reserpine also affects mast cells in this way, dilutions of 1 : 6,000 in physiological saline causing loss of basophilia in 2 min. Higher dilutions take longer to accomplish this effect and dilutions between 1 : 12,000 and 1 : 24,000 require about 45 min. Exposure to light has only a small enhancing effect.

The next step in this investigation is to determine the nature of the basophilic substance of mast cell granules and its relationship to 5-hydroxytryptamine.

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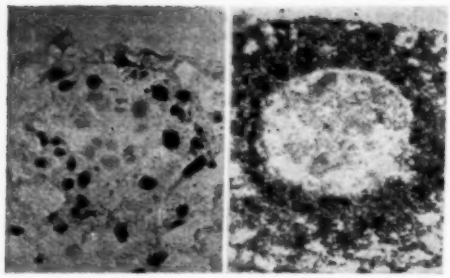
Failure of Nucleotides to diffuse freely into the Embryo of *Pteridium aquilinum*

In the ferns the embryo develops within the gametophyte and is nutritionally dependent on it until the first root and leaf emerge. In the homosporous leptosporangiate ferns the period of complete nutritional dependence is at least ten days and is usually longer. Despite the fact that the young embryo is in intimate physical contact with the gametophyte during this period, the results of experiments described here suggest that there may, nevertheless, be incomplete metabolic union between the two generations.

Gametophytes of *Pteridium aquilinum* containing young embryos were placed on media containing tritiated thymidine and tritiated uridine. The experimental technique has already been described[1], and the gametophytes were fixed four days after the feeding with the labelled nucleotide.

The autoradiographs show that although radioactivity is concentrated around the embryo there is little if any in the embryo itself. This is particularly clear with the gametophyte fed with tritiated thymidine (Fig. 1*A*), a nucleotide which would enter only the deoxyribonucleic acid. The gametophytic nuclei adjacent to the embryo are heavily labelled and appear black in the autoradiograph, but those of the embryo, despite the fact that nuclear divisions are occurring, are entirely unlabelled. The few grains scattered over the embryo can be put down wholly to background radioactivity. These results are in striking contrast with those of earlier experiments[1] in which it was shown that tritiated thymidine was able freely to enter developing and unfertilized eggs. In the gametophyte fed with tritiated uridine, which would enter only the ribonucleic acid, the cytoplasm of the cells around the embryo is clearly much more heavily labelled than that of the embryo (Fig. 1*B*). The frequency of the grains above the embryo is about four times the background, and about a quarter of that of the grains above the cytoplasm of the cells not adjacent to the embryo. Above the cells around the embryo, which have copious cytoplasm and every appearance of metabolic activity, the grains are too dense to count. Thus it appears that only a small proportion of the radioactivity entering the gametophyte with the uridine reaches the embryo.

XUM



A *B*

Fig. 1. Autoradiographs of embryos of *Pteridium aquilinum* after feeding the gametophyte with tritiated nucleotides. *A*, 32-celled embryo, approximately 10 days old at time of fixing. Gametophyte fed with tritiated thymidine. *B*, Larger embryo, probably about 15 days old at time of fixing. Gametophyte fed with tritiated uridine. (× 200)

These results indicate that at least two nucleotides cross the boundary between gametophyte and sporophyte with difficulty or not at all. On general principles of permeability, it would be expected that other molecules of similar or larger size would also fail to cross this boundary. In view of the clear-cut result with thymidine, it may be that the appearance of some radioactivity in the embryo when labelled uridine is fed results not from its entering the embryo directly but from catabolism of some of the uridine in the metabolically active cells adjacent to the embryo, and the passage into the embryo of simpler derived molecules, such as orotic or even aspartic acid. There is a strong possibility, therefore, that the sporophytic embryo draws only simpler molecules from the gametophyte, and that the sporophyte has much metabolic autonomy *ab initio*. This would explain why Jayasekera and I[2] were able to grow normal sporophytes from very young embryos of *Thelypteris*, surrounded by only small amounts of gametophytic tissue, placed on a simple mineral-agar medium.

That the cytoplasm of the fertilized egg comes away cleanly from the wall of the archegonium on fixation, whereas that of the unfertilized does not, suggests that the change in the bounding layer of the cytoplasm leading to the separation of the gametophytic and sporophytic metabolisms occurs on fertilization. Possibly the alteration in the surface of the egg which is believed to prevent polyspermy also results in a permanent alteration in its permeability to large molecules.

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Sexual Differentiation in *Hydra* in Relation to Population Density

Recent reports by Loomis and by Loomis and Lenhoff[1] of controlled induction of sexual differentiation in *Hydra littoralis* have stimulated considerable interest in this process. One of the observations reported is that hydras differentiate sexually in crowded cultures but remain asexual when uncrowded unless the cultures stagnate. This observation is of particular interest because mass cultures of the clone of *Hydra littoralis* used by Daniel and Park and by Park[2] remained continuously asexual for as long as three years. Although culture conditions were considerably different from those described by Loomis, population density and degree of stagnation were apparently comparable.

The Daniel–Park clone of hydras is no longer in existence. However, the present mass cultures in this Laboratory, derived from a single male of Loomis' stock, and maintained for two years according to the methods described by Loomis and Lenhoff, have exhibited cycles of sexuality in both sparse and crowded cultures[3]. It therefore seemed desirable to examine more critically the relation between population density and sexual differentiation.

Hydras were cultured in Loomis' bicarbonate–versene-tap water medium (BVT) at 24° ± 2° C. Daily except Sunday the cultures were fed on brine shrimp larvæ in excess for ½ hr., rinsed with tap water and re-covered with BVT medium. Concurrently the numbers of hydras with and without gonads were recorded. Two sets of observations were made, one comparing the percentage of hydras with gonads in mass cultures of differing population densities, and the other dealing with the occurrence of gonads in isolated individual hydras.

In the mass population comparisons, a group of randomly chosen hydras in a container giving a population density of 2·5 hydras per ml. of medium served as a control. The culture was maintained at 100 by daily random thinning for 217 days. It was compared with a culture of 100 asexual hydras in a container such that the population density was originally 0·67 hydra per ml. of medium. This experimental culture was followed through the following four successive population régimes:

(1) Allowed to increase to 400 individuals (attained on day 11) and thereafter maintained at 400 (density 2·67 individuals per ml.) by daily random thinning for 34 days; (2) immediate repetition of (1), 400 individuals being reattained on day 65, and the population being at 2·67 individuals per ml. for 24 additional days (total days from set up of culture, 89 days); (3) culture then reduced to 100 individuals (density 0·67 individual per ml.) and maintained at this level for 92 additional days; (4) all but 20 males with gonads discarded (density 0·13 individual per ml.) and these individual hydras maintained, all buds being discarded as soon as dropped for 52 additional days (total 233 days). The records for this experiment show that although there were transitory drops in percentage of gonad-bearing hydras in the experimental culture each time its population density was reduced, the two cultures were strikingly similar in the time sequence of sexuality and asexuality. Both cultures concurrently went through several periods, one of nearly 30 days, in which not a single individual had gonads, and both also went concurrently through several periods when 35–80 per cent of the hydras bore gonads.

In the second set of observations, 24 male hydras with gonads were transferred from the stock into individual culture dishes of a size such that the population density was 0·1 hydra per ml. of medium. Each hydra was fed, washed and re-covered with medium, as with the mass cultures, and newly dropped buds were removed within 24 hr. The hydras were kept on this régime for 86–180 days.

All the hydras became asexual after 4–13 days of isolation. However, all again became sexual within an additional 2–23 days. Thereafter, several more

asexual–sexual cycles of varying lengths occurred. Extreme variability in the length of sexual and asexual periods was in fact an outstanding characteristic. Asexual periods varied in length from one to 97 days; sexual periods varied from one to 103 days.

In summary, although we cannot at present explain the alternating periods of sexuality and asexuality which occur in mass and isolation cultures and at population densities ranging from 0·1 to 2·7 individuals per ml., it is clear that neither crowding nor stagnation is necessary for sexual differentiation to occur.

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The Number of Testis Follicles in Miridae

THE male reproductive system of bugs has been investigated in detail for a handful of species[1] or, in a range of species, quantitative data presented on the testis follicles[2]; but the dominant family in temperate climates, Miridae, has scarcely been examined—probably because of the difficulty specific determination presents to non-specialists.

This communication summarizes details of counts made of testis follicles in 90 of the 199 mirids listed as British[3]. Where the follicles were small, convoluted or otherwise obscured aceto-orcein staining, as in routine bug cytology, was used to aid visualization[4]. The seven species seen by Woodward[2] were re-examined.

The non-British Cylapinae and Palaucorinae—the latter of dubious status and monotypic—make up, with the six sub-families listed in Table 1, the family Miridae, a family the limits of which are not in dispute. Kullenberg believed that, as a rule, mirids have seven follicles in each testis, but this is contradicted by my work. From Table 1 it is seen that follicle numbers range from one to eight, while all sub-families in which five or more species have been examined exhibit a marked modal number, namely: Phylinae 3, Dicyphinae 1, Orthotylinae 2 and Mirinae 7. Such distinction between the groups was unexpected: there are no cases within the Arthropoda, so far as is known, in which this apparently fundamental structure, one in which it is difficult to envisage selective advantage in any of the variants, exhibits such division into compartments at sub-family level.

Table 1. TESTIS FOLLICLE NUMBER IN 90 BRITISH MIRIDS

Sub-family	Species	Follicles
Bryocorinae	*Monalocoris filicis*	2
Deraeocorinae	*Alloeotomus gothicus*	3–4 variable
	Deraeocoris lutescens	2
	D. olivaceus	7
	D. ruber	8
Phylinae	23 species	3
	Oncotylus viridiflavus	4–5 variable
Dicyphinae	5 species	1
Orthotylinae	20 species	2
	Cyrtorhinus caricis	1
	Orthocephalus coriaceus	4?
Mirinae	30 species	7
	Notostira elongata	3
	Leptopterna dolabrata	8
	L. ferrugata	8
	Stenotus binotatus	8

Discussion of the bearing of these facts on the phylogeny of Miridae must await a re-assessment of all the structures now investigated in the family: male and female genitalia[5], chromosome numbers[4], ovariole number[6] and eggs[7], in addition to the traditional 'characters' of the systematist (arolia, pseudarolia, wing venation, pronotal structure, etc.). The results do, however, suggest: that Dicyphinae warrants sub-family status, as has been suggested elsewhere on other grounds[4]; that intraspecific variation—which is accompanied by unilateral variation—is a rare phenomenon, only noted in two of the 90 species examined; that in Orthotylinae the position of certain Halticini, here represented by *Orthocephalus coriaceus*, requires investigation, as suggested on other grounds[5]; that *Cyrtorhinus* may possibly be wrongly placed at present; that it is doubtful whether *Deraeocoris lutescens* on one hand and *D. olivaceus* and *D. ruber* on the other are congeneric; that a follicle number of eight occurs only in groups in which seven is perhaps the usual number.

The diverse results obtained from the four deraeocorines, and that for the single bryocorine, mean little at present; both groups are essentially tropical. The clearest case of wide divergence from a well-defined sub-family pattern is that of *Notostira elongata*, a bug the systematic position of which has never been questioned.

The full list of species will be published elsewhere.

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Evolution of the Sweet Potato (*Ipomoea batatas* (L.) Lam.)

RECENT work on meiosis[1,2] has given considerable support to the suggestion of King and Bamford[3], based on mitotic investigations, that the sweet potato is an allopolyploid, $2n = 90$, resulting from hybridization and natural doubling of the chromosome number in $F1$. The incidence of secondary associations of bivalent chromosomes at metaphase has been interpreted by Ting *et al.*[3] as indicating that the sweet potato, derived from somewhat related species, is a 'relatively recent species'.

While this interpretation may be of general validity in sexually reproducing plants, its unqualified applica-

tion in this case may be challenged in respect of the known mode of reproduction of the sweet potato. The plant is propagated vegetatively throughout the areas of its cultivation. In present research under a grant from the Rockefeller Foundation, field collections have been made in Peru, Bolivia, Ecuador, Colombia, as well as in the Pacific Islands, Polynesia and Melanesia. In no case was purposeful propagation by seed recorded either in practice or by local tradition. In Peru and Colombia seeds were collected from cultivated crops, and although informants were aware of the occurrence of these in both coastal and marginal jungle areas, no evidence of their use was found. No wild prototypes of the sweet potato have been found, so that it seems likely that the method of propagation may have existed since very early times, possibly since the plant's domestication. In discussing the likelihood of a South American domestication, Sauer[4] places the sweet potato in "a culture complex that operated by vegetal means of reproduction . . . not by seed production".

The conservative breeding system of the plant, accentuated by the selective activity of man, thus retards the rate of evolution of this species. While chromosome behaviour in meiosis does indicate that in this respect the species has not reached maturity, this behaviour cannot be used as a time-device to signify the age of the species. It is conceivable that chromosome multivalents could have been perpetuated for as long as man has cultivated the plant.

Ames[5] has delineated a sequence of agricultural development that passed through successive horticultural and agricultural phases, an early stage in the former being the use of vegetative reproduction of plants. If this sequence is accepted, the history of some present-day sweet potato clones may have anteceded that of grains in America.

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Growth of Fern Embryos in Sterile Culture

In recent years, advances in the field of embryo culture have made possible the successful growth of a number of plant species which would not otherwise have been propagated[1]. These cultural experiments have also enriched considerably our understanding of the physiological and morphological aspects of early plant development[2,3]. Even though considerable information is available from this work on the cultural requirements of embryos, up to the present time it has not been possible to maintain very young, globular plant embryos (30–85μ) *in vitro*[4].

For the most part, experiments have been conducted utilizing embryos from both gymnosperms and angiosperms, and relatively little consideration has been given to embryos of the ferns and mosses. One of the major reasons for neglect of these plants in embryological experiments has been technical and manipulative difficulties of approaching their embryos, for they are formed and develop in the venter of the archegonium surrounded by prothallial tissue. That these difficulties are not insurmountable can be seen from the work initiated at the Harvard Laboratories several years ago by Ward and Wetmore[5], and more recently elsewhere[6,7]. Although Ward and Wetmore, utilizing surgical techniques on the fern *Phlebodium* (*Polypodium*) *aureum* J. Sm., were able partially to free the embryo from the surrounding tissue, they were unable to isolate it completely as it appeared to be attached to the wall of the venter.

In the course of work on the life-history and embryology of the fern *Todea barbara* (L.) Moore[8], it was found that the embryo was not demonstrably adherent to the venter in at least the younger stages of embryo formation. It could be removed easily from the surrounding prothallial tissue without apparent damage. The ease with which quantities of embryos of all ages, 4–20 days following fertilization, could be excised prompted the initiation of work designed to determine whether fern embryos could be grown to maturity in sterile culture when completely removed from the parent prothallus.

Sexually mature prothalli previously grown under sterile conditions on a simple nutrient medium[9] were immersed in sterile water for 3 hr. in order to facilitate fertilization. After the water was removed, the prothalli were watched for growth of embryos which were characteristically numerous. Those utilized in this work were removed aseptically from the prothalli according to the procedure outlined in a previous report[10]. Cultural experiments were conducted, employing a liquid medium consisting of Knudson's solution of half-strength to which was added 10 gm. of sucrose, 10 mgm. ferric citrate, and 1 ml. of trace element solution per litre of nutrient solution[11]. The trace element solution was modified by the addition of 25 mgm. cobaltous chloride per litre. In this basic medium, isolated twenty-day-old embryos (240–260μ) possessing stem and root meristems were grown to advanced sporelings in one month. When later planted in the soil, these young plants give every assurance of becoming adult plants, capable of fruiting. Slightly younger embryos, isolated 17 days after fertilization (160μ), showing an absorbing foot but no other obvious signs of organization, required the addition of 10 per cent autoclaved coconut milk to achieve continuing growth and development. Succeeding experiments have demonstrated that the requirements of coconut milk for 17-day-old embryos cannot be replaced by any known concentrations of varied amino-acids, but a mixture of 50 mgm./l. each of sorbitol and inositol, components of the 'neutral' fraction of coconut milk[12], will do so. Very young, unicellular embryos isolated 4–5 days after fertilization (35–55μ), before the first division of the embryo, can also be maintained in the simple basic medium when supplied with 3 per cent sucrose and 50 mgm./l. of sorbitol and inositol. The first few divisions in the post-fertilized egg stage of young cultured embryos are formed in essentially the same planes as would ordinarily be the case with such young embryos growing in their natural environment of the prothallial-contained archegonium. Succeeding divisions, however, appear to be oriented haphazardly, giving rise to embryos which, even after one month in culture, displayed little evidence of the usual pattern of development. Instead, they develop into irregular, asymmetrical, non-uniform, thalloid plants which do not become vascular, do not develop leaves and roots, but do remain associated closely with the nutritive medium, much as do prothalli. Whether

these prothalloid embryos can be altered so as to develop into the usual erect, vascular leaf- and root-bearing *Todea* fern plants is part of the work now being continued.

The results reported here demonstrate that embryos of at least one vascular plant, the fern *Todea barbara*, can be removed from their enclosing archegonial venters and maintained successfully outside the plant at all stages of their development on an artificial nutrient medium. If they are removed after they have become compact, spherical aggregations of cells which have already established nutritional contact with the adjacent prothallial tissue by an absorbing foot, and have already acquired an initial leaf and a root primordium (20 days or older), they will grow into fern plants *in vitro* on a simple medium of mineral salt with a low content of sugar. They are also capable apparently of synthesizing their own hormonal and other organic needs. If they are removed when slightly younger (17 days or less) when they have already become somewhat spherical organisms, each with an absorbing foot but no appendages as yet, and are grown on the medium mentioned but supplemented by coconut milk, they develop into the usual embryos characteristic for *Todea*. Appropriate concentrations of sorbitol and inositol in place of coconut milk produce comparable embryos in approximately the same time. If, however, very young embryos are removed 5–6 days after fertilization, but before the first cell-division occurs, and if they are cultured on either of the agar-enriched nutrient media just mentioned, the embryos develop, but not into spherical organisms. Rather, they become prothalloid in their flattish, extending form until they are spreading, irregular, often branching entities—no two look alike—in close and intimate contact with the nutritive medium. They have not as yet acquired appendages, nor apical meristems nor vascular tissue.

This work suggests that in the early stages of its development the embryo depends on an external source for formative substances, the prothallus. A suggestion from which to start further work might appropriately indicate that the necessary enzymes have not yet been synthesized in very young embryos. But of equal importance, and too often neglected, is the influence of environment on these young embryos. Even when seeming to be nourished adequately, the freed embryo resembles much more the derivative growth of the freely germinating spore—the prothallus. When it develops in the containing archegonial venter, with its turgid cells, the young embryo follows a much more regularized pattern. In this environment, it develops into a spherical entity which ultimately—certainly by the seventeenth day—establishes direct, broad, nutritional contact with its neighbouring prothallial cells. When it is isolated, it clearly acquires the ability to synthesize its own biochemical needs, presumably including its own enzyme systems.

We are also forced to believe that in the venter the planes of cell division in the young embryo are so oriented as to produce a spherical organism. This end-product is clearly not produced in early, excised embryos. If mitotic spindles are formed in specific orientations in the cell under restraints, or pressures, then one could understand better early shapes and organizations of embryos. Nutrition alone does not seem an adequate explanation.

Techniques of embryo isolation and culture provide the possibility of conducting investigations into the various physiological and morphogenetic mechanisms which control the growth and development of plants. The correlations between free and contained development and the orientation of mitotic spindles with consequent cell-plate and cell-wall formation and their relations to shapes of early embryos can now be subjected to electron microscope work and possible subsequent interpretation. Moreover, the accumulating evidence from single-cell cultures must be taken into consideration.

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Physiological Significance of Surface Wax on Leaves

A STUDY of the surface wax occurring on the leaves of New Zealand and Spanish white clovers (*Trifolium repens*) has been made with the electron microscope. Pre-shadowed carbon replicas were prepared in a manner similar to that used by Bradley[1], and it was found that the wax had a structure characteristic for each species and was extruded from the cuticle only while a leaf was growing. This work was later extended to include Montgomery, Broadleaf and Moroccan red clovers. During the investigation it was noted that wax was removed by weathering, for example, contact between leaves and between leaf and ground in windy conditions, and that the weathered appearance could be simulated by brushing the leaves with a camel-hair brush. In both cases the wax was removed from the crown of the dome-shaped cuticle overlying the epidermal cells. Fig. 1 shows an adaxial surface of New Zealand white clover before brushing and Fig. 2 after brushing.

To supplement this observation, in particular to provide a ready measure of the quantity of wax, the contact angle of water droplets on the leaves was measured with a microprojector[2]. Removal of wax by brushing or weathering always lessened the angle of contact.

The contribution of wax to the water balance of the plant was assessed by measuring the transpiration-rate of excised leaves using the technique described by Hygen[3]. Pairs of leaves of the same age and size were brought to full turgidity, and losses of weight recorded under fixed conditions. Fig. 3 shows a semi-logarithmic plot of weights against time, typical of brushed and unbrushed leaves. By extending the weighings over a number of hours it was found that

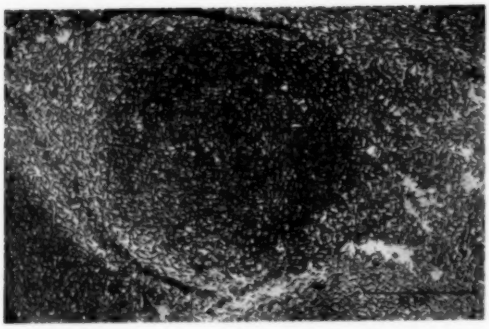

Fig. 1. Surface structure over a single epidermal cell showing undamaged waxy coating. Direct print from a micrograph

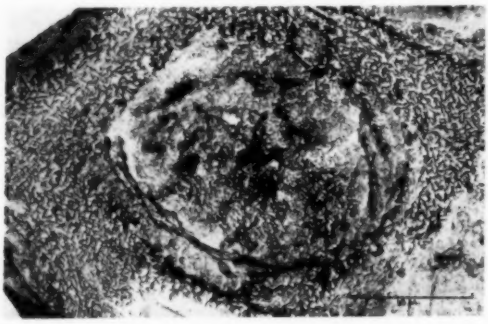

Fig. 2. Leaf surface of similar area to Fig. 1, showing removal of wax particles from dome

the increased transpiration of the brushed leaves was due to greater loss during the cuticular phase, that is, principally after the stomata had closed.

It is suggested here that when growth is interrupted by adverse conditions, the wax that is removed by weathering is not replaced quickly enough and the plant is additionally stressed through having an unusually high cuticular leaf transpiration. This is borne out by observations in the field.

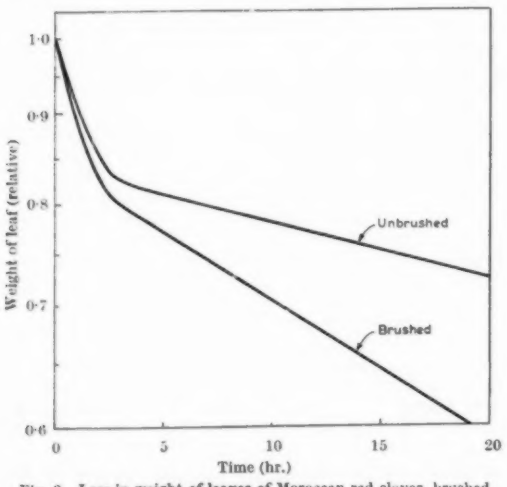

Fig. 3. Loss in weight of leaves of Moroccan red clover, brushed and unbrushed, versus time

Some evidence was also obtained suggesting that the presence and form of wax on leaf surfaces are related to frost hardiness.

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VIROLOGY

Unusual Base Composition of the Ribonucleic Acid of Wild Cucumber Mosaic Virus

At the present time the small viruses containing ribonucleic acid (RNA-containing viruses) seem to be useful test objects for discovering the relationship between the amino-acid sequence of proteins and the structure of the RNA which is presumed to code that sequence. It is believed that the RNA of the virus specifies the virus coat protein. In addition, it perhaps carries the code for other proteins and/or plays some structural part. We wish to point out that the RNA from wild cucumber mosaic virus has an unusual base composition, that relatively small pieces of it are obtainable and that these attributes may be useful in the solution of the coding problem.

Wild cucumber mosaic virus has a molecular weight of about 7 million and an RNA content of about 2·4 million[1]. Plants infected with the virus produce two virus-like macromolecular particles (designated T_a and T_b) in addition to the virus. Various physical and chemical tests[1,2] have shown that these particles and the virus are similar (or identical) in so far as their protein portion is concerned, but that they differ in their content of RNA. $T_a (M = 4 \times 10^6)$ contains no RNA, T_b $(M = 4{\cdot}4 \times 10^6)$ contains about $0{\cdot}4 \times 10^6$ molecular weight units of RNA. The relative proportion of nucleotide residues in the RNA from the virus and from the T_b particle are similar but perhaps not identical. Both have an unusually high proportion of cytosine (see Table 1), comparable to the high proportion found in turnip yellow mosaic virus[3].

The fact that the composition of the RNA of T_b is unusual and at the same time similar to that of the virus RNA suggests that it has virus-like nucleotide sequences. In any event, its composition is different from that of ribosomes from small sugar pumpkin (see Table 1), the host on which wild cucumber mosaic virus was grown. However, the RNA of T_b cannot be identical with the virus RNA since the latter is a packet about six times as large. Possibly the virus RNA has similar or identical sub-units, one of which is present in each T_b particle. Alternatively, the individual T_b particles may contain different portions, but similar quantities, of the virus RNA. Thus only on the average would the T_b base composition be similar to that of the viral RNA.

Even though proteins other than the protein of the virus coat may be coded by the RNA of wild cucumber mosaic virus, it might be anticipated that the unusual base composition of the latter would be reflected in an unusual amino-acid composition for the protein of the coat. Evidently this is not so. The amino-acid composition is recognizably—but not startlingly—different from that

Table 1. Molar Base Composition of RNA in Wild Cucumber Mosaic Virus and in the T_b Particle of the Virus
By way of comparison, we show also the base composition of the RNA in ribosomes of uninfected sugar pumpkin (the host on which wild cucumber mosaic virus was grown) and also the base composition of the RNA in tobacco mosaic virus

	Adenine	Guanine	Cytosine	Uracil
Wild cucumber mosaic virus*	18·1	16·5	39·8	25·6
	18·5	15·3	40·2	26·0
„ „ T_b*	19·5	20·3	35·3	24·9
	18·9	17·8	37·8	25·5
Ribosomes of sugar pumpkin*	23·7	32·8	23·8	19·7
	23·4	33·8	23·4	19·4
Tobacco mosaic virus	30·0	25·0	18·5	26·5

* Duplicate experiments by the method of Smith and Markham (ref. 4).

Table 2. Amino-acid Composition of the Protein of Wild Cucumber Mosaic Virus, and for Comparison the Amino-acid Composition of Tobacco Mosaic Virus

	Wild cucumber mosaic virus*	Tobacco mosaic virus¶
Alanine	5·3	5·6
Ammonia	2·0	1·8§
Arginine	3·8	9·7
Aspartic acid and asparagine	7·8	11·7
Cysteine (or half-cystine)	0·9	0·6
Glutamic acid and glutamine	6·6	11·7
Glycine	2·6	1·9
Histidine	2·0	0·0
Isoleucine	7·2	5·1
Leucine	13·0	7·7
Lysine	5·8	1·4
Methionine	0·7	0·0
Phenylalanine	6·0	6·7
Proline	9·2	4·4
Serine	9·9†	7·9
Threonine	5·8†	9·1
Tryptophan	1·7‡	3·2
Tyrosine	3·1	3·7
Valine	6·6	7·8

* Analysed by means of the method of Spackman, Stein and Moore (ref. 6); hydrolysis of the protein was carried out for 24 hr. at 110°.
† Corrected for an assumed loss of 6 per cent in the case of serine and 11 per cent in the case of threonine.
‡ Determined by the method of Goodwin and Morton (ref. 7).
§ Calculated from the amino-acid composition given by Wittmann (ref. 8).
¶ From Ramachandran (ref. 9).

of the coat protein of tobacco mosaic virus, the RNA of which has quite a low proportion of cytosine (18 per cent, see Table 1). It will be seen from Table 2 that of the 15 amino-acids present in greatest abundance in wild cucumber mosaic virus and tobacco mosaic virus, only in two instances, arginine and proline, does the relative abundance differ by more than a factor of two. The situation is not greatly different if the comparison is made instead with bushy stunt virus, which also has a relatively low content of cytosine (22 per cent, ref. 3).

It might be interpolated at this point that, especially in the case of bushy stunt virus, the amount of protein codable by the virus RNA is not large. Coding theory considerations suggest that at least three nucleotide residues would be required to code each amino-acid[5]. Very likely the codes found in Nature would be less efficient than this theoretical value. Thus from a knowledge of the nucleic acid content and the molecular weight of the protein sub-unit[10] of bushy stunt virus, it is apparent that less than 1,600 amino-acids can be coded, about 600 of which are required for the bushy stunt virus sub-unit. For tobacco mosaic virus the corresponding numbers are 2,100 and 150. By a similar computation it can be shown that less than 2,500 amino-acids are codable by the RNA of wild cucumber mosaic virus, about 200 of which are required by the protein sub-unit (the molecular weight of which is about 20,000, ref. 1)). The RNA from wild cucumber mosaic virus T_b could code no more than 450 amino-acids.

If all the nucleotide residues in the RNA's of wild cucumber mosaic virus, tobacco mosaic virus and bushy stunt virus participate directly in coding, then codes in which one particular sequence of nucleotides determines a particular amino-acid would seem to be unlikely. In order for such a code to hold, a 40 per cent cytosine content in wild cucumber mosaic virus RNA and an 18 per cent content in tobacco mosaic virus RNA would require a very distinctive difference in the relative abundance of the amino-acids coded in the two cases. On the average, those amino-acids in the specification of which cytosine participates would have to be more than twice as abundant in the case of wild cucumber mosaic virus. If the frequently discussed 4-symbol, comma-less, non-overlapping code[11] holds, twelve amino-acids would have to have this high average abundance. Since such differences are not evident in the coat proteins, even larger differences would be required in any other proteins that conceivably are coded.

A more plausible alternative is that a considerable fraction of the nucleotide residues plays a part different from that of participating in direct specification of particular amino-acids. Perhaps they serve as spacers or as structural elements.

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Mechanism of Recovery from Viral Infection in the Chick Embryo

The chick embryo lacks many of the mechanisms which are thought to be required for recovery from viral infections. Production of antibody and delayed hypersensitivity (as measured by ability to reject foreign tissue) do not occur in the chick embryo of any age[1,2], whereas the interferon mechanism begins to function in the embryo at about eight days and increases thereafter[3]. If interferon plays a major part in recovery from viral infection[4,5], then a correlation would be expected between the lethality of viral infections and absence of the interferon mechanism in young embryos. Decreasing mortality with increasing age of embryos has been reported with a number of viruses[6,7]. However, the cells of these variously aged embryos yield equal amounts of virus[3,8]. The present work was undertaken to determine whether the appearance of the interferon mechanism during development of the chick embryo is parallel to increased ability to recover from viral infection.

Groups of ten chick embryos, aged 4, 7, 10, and 13 days, were infected with each of four different

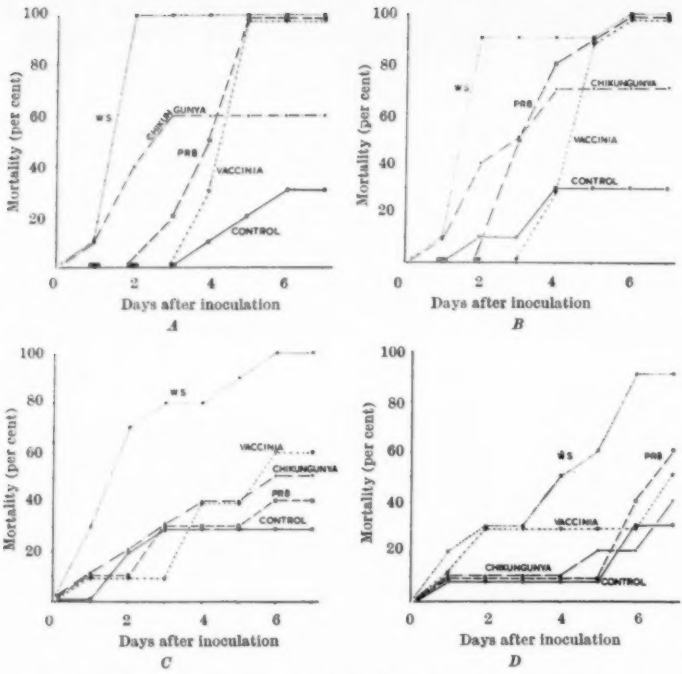

Fig. 1. Mortality following injection of four viruses into chick embryos of different ages. Eggs: *A*, 4-day; *B*, 7-day; *C*, 10-day; *D*, 13-day

viruses. Inoculations of 0·05 ml. containing 10^4–10^5 infectious doses of vaccinia, Chikungunya (Arbor virus group *A*), or influenza (*WS* and *PR* 8 strains) viruses were made into the yolk sac. Controls were injected with broth diluent. The eggs were then candled daily to determine viability of the embryos. Eggs containing embryos, which were non-viable on candling, were opened to confirm death and all embryonated eggs which survived for seven days were opened to confirm viability.

As shown by the graphs in Fig. 1 the mortality-rate of embryos decreased as the age of embryos increased. The lethality among 4- and 7-day embryos, infected with vaccinia, Chikungunya or *PR*8 viruses, was high, whereas the mortality among 10- and 13-day embryos tended to be close to that of the controls. The more virulent *WS* virus caused early deaths in young embryos, but delayed deaths in older embryos.

Fig. 2 summarizes the effect of the age of the chick embryo on its *in vitro* sensitivity to the antiviral action of interferon and on its ability to survive infection with these four viruses. Between the seventh and tenth days there was a rapid development of sensitivity to the antiviral action of interferon and an equally rapid increase in the ability to recover from viral infections. The close correlation of enhanced recovery of embryos with development of the interferon mechanism is consistent with previous findings which related recovery from viral infection of mice to production of interferon[4,9].

Evidence indicating that antibody is not essential for recovery from many viral infections has been reviewed[10]. In the present work survival of embryos was enhanced in the presence of a functioning interferon mechanism[3] and in the absence of antibody formation or delayed hypersensitivity[1,2], suggesting that interferon alone may be an essential factor in recovery from many viral infections.

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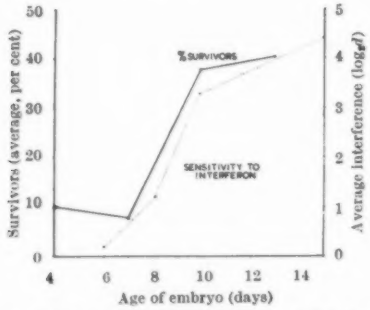

Fig. 2. Effect of age of chick embryo on its ability to survive infection and on its sensitivity to the antiviral action of interferon *in vitro*. Survival in each group was determined by averaging the percentage of embryos surviving 7 days after infection with the four viruses (Fig. 1). Sensitivity to the antiviral action of interferon was determined from the results of a previous study (ref. 3) by averaging the sensitivity of each age group at three doses of interferon

GENETICS

Basis of the Diploid-like Meiotic Behaviour of Polyploid Cotton

The cultivated cottons (*Gossypium barbadense* and *G. hirsutum*) are allotetraploids derived from two geographically isolated diploid species; one, an Asiatic species, donating the A genome and the other, an American species, donating the D genome. The chromosomes of the diploid progenitors differ in length, those of the A genome species being longer than those of the D genome species. Furthermore, this size difference is maintained in the polyploid, in which chromosomes of two size-categories can be recognized. A histogram (Fig. 1) of the lengths of the

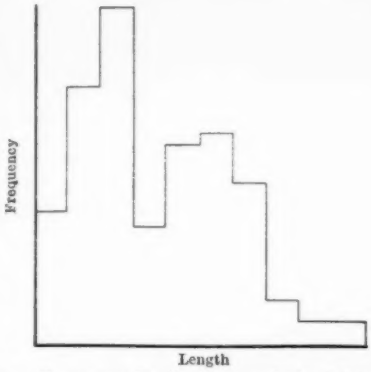

Fig. 1. Chromosome lengths in seven plants of *G. barbadense*

somatic chromosomes from root-tips of seven plants of *G. barbadense* shows a bimodal distribution of the chromosome lengths and also indicates that there is a small overlap between the two groups.

Several workers[1] have employed this differential in chromosome length to categorize meiotic configurations in interspecific hybrids. The difference in size can also be used to recognize the univalent chromosomes seen at meiosis in haploids of the tetraploid species. Chiasma formation occurs infrequently in these haploids, but the univalents often associate in pairs as though synapsis had occurred but no chiasmata had been formed. These associations can be classified into three types depending solely on the lengths of the chromosomes involved; first a long chromosome associated with another long (abbreviated to *LL*), secondly a long chromosome with a short (*LS*) and finally a short with a short (*SS*).

If the chromosomes were associating with each other randomly, and since there are equal numbers of long and short chromosomes, then the numbers of *LL*, *LS* and *SS* associations should be in the ratio 1 : 2 : 1. Table 1 shows the totals of each class of association from 124 cells that were analysed from five haploids of *G. barbadense*. These figures, when tested against the hypothesis of random association, differ significantly ($P<0{\cdot}001$, $n=2$) from the expectation; the *LS* associations being considerably in excess. Also it can be shown that since the frequencies of the *LL* and *SS* groups are approximately equal, then the inflation of the *LS* class cannot be due to the consistent misclassification of long for short chromosomes or vice versa.

Table 1. Association of Long and Short Chromosomes at Meiosis in Haploids of *G. barbadense*

Association	*LL*	*LS*	*SS*	Total
Observed	65	202	62	329
Expected (1 : 2 : 1)	82·25	164·5	82·25	329

$\chi^2 = 17{\cdot}15$. $n = 2$. $P < 0{\cdot}001$

Since it is plausible that the A and D genome diploids diverged from a common archetype and since duplicate loci have been demonstrated frequently in the allotetraploids, the chromosome association observed in these haploids probably results from a basic similarity of the two genomic sets. In the tetraploid, however, the chromosomes only form bivalents, so that if chromosomes of the different genomes are as similar as these results would indicate, it is probable that there is a genetic system of diploidization in cotton, analogous to that in wheat, organizing the meiotic behaviour so that only homologous and not homœologous chromosomes can pair.

The identification of the chromosome, and the particular chromosome arm, involved in the cytological diploidization of wheat is only possible because of the complete range of aneuploids available in that species[3]. In the absence of a similar range of aneuploids in cotton a precise identification of a genetically controlled mechanism cannot be made. However, by a consideration of certain hybrid combinations, further corroborating evidence can be adduced indicating the existence of genetic diploidization in cotton. The pertinent data are given in Table 2.

Table 2. Analyses of Meiosis in the A and D Genome Combinations in *Gossypium*

Hybrid or haploid	Genome	Chromosome pairing	Author
Triploid	A × AD	13 I, 9 II and 2 IV	Gerstel (ref. 5)
Triploid	D × AD	13.00 I, 12.15 II, 0.51 III and 0.04 IV	Endrizzi (ref. 1)
Diploid	A × D	9.6 I, 7.8 II, 0.2 III and 0.05 IV	Skovsted (ref. 1)
Haploid	(AD)	26 I	Kimber

The chromosomes of the A genome diploids pair with the long chromosomes of the AD allotetraploids in hybrids, leaving 13 short univalents. Similarly, the chromosomes of D genome diploids pair with the short chromosomes of the AD allotetraploids and there are 13 long univalents (Table 2). In this way the cytological equivalence of the diploids to their respective genomes in the tetraploids was shown. A similar, and considerable, cytological equivalence between the two diploids is also shown by the high mean synapsis observed in the AD hybrid *G. arboreum* × *G. thurberi*.

These relationships can be expressed diagrammatically (Fig. 2) when it is immediately apparent that an incongruity exists. If the A genome of the diploid is equivalent to the A genome of the tetraploid, and the D genome of the diploid similarly equivalent to the D genome of the tetraploid, and simultaneously the D diploid is equivalent to the A diploid, then the AD tetraploid should have a cytological behaviour characteristic of an autotetraploid. Since the tetraploid cottons are regular bivalent formers, it is most reasonable to suppose that a genetical change has

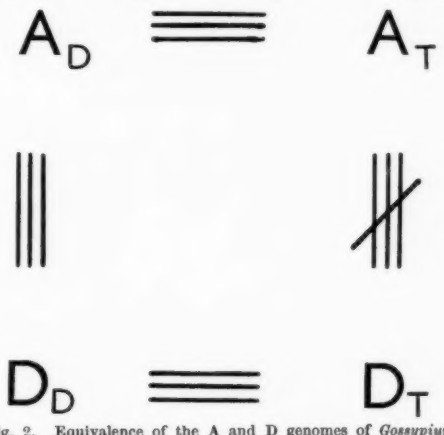

Fig. 2. Equivalence of the A and D genomes of *Gossypium*. The subscripts indicate diploid (*D*) and tetraploid (*T*)

occurred, since the inception of the polyploid, such that pairing is restricted to homologous chromosomes only.

This method of recognizing the genetical control of the cytologically diploid behaviour of allopolyploids can be applied to *Nicotiana tabacum*. Again it is easy to demonstrate the equivalence of the diploid genomes both to their respective genomes in the allopolyploid and also to each other, while the polyhaploid of *N. tabacum* shows almost no pairing.

The effective isolation of genomes in allopolyploids, when the same genomes can pair in diploid hybrids, provides a generalized method for recognizing the genetical control of the diploid-like behaviour of the allopolyploids. Theoretically, the method is applicable to any allopolyploid, but it is difficult in practice to accumulate sufficient data to make a complete cycle of comparisons. Because of the generalized nature of the method, it lacks the precision of the situation in wheat where a single chromosome arm can be held responsible for the effect. However, the method is applicable to all cases where the parents and a haploid of the allopolyploid are known and therefore can be used, even when a complete range of aneuploids is not available.

It is not possible in cotton, at the present, to obtain information on the mode of action of the genetical diploidization, and it would be unwise to assume, *a priori*, that the functioning is identical to that in wheat. However, with the development of a monosomic series in cotton more precise information may be obtained.

Other allopolyploid species may also have become diploidized by genetical processes and not by the slow accumulation of many small structural changes in the chromosomes. The efficiency of a single-step change, if the diploidization is governed by a single gene, to regular meiotic behaviour presents an attractive hypothesis. This hypothesis is conceptually simpler than that required to explain the vague and randomized processes of the addition of small chromosomal changes to already existing alterations. The acceptance of the idea that allopolyploids other than wheat have become diploidized by genetical processes does not mean that some species may not have a regular chromosome behaviour that is governed solely by differential affinity[4]. However, it is worth while considering that, for an allopolyploid to be formed, first of all an F_1 hybrid must be made. The occurrence in Nature of an F_1 hybrid implies some similarity of the two parental species. If the species are so similar that they are able to hybridize, then their chromosomes will rarely be so dissimilar that pairing is entirely autosyndetic in the derived allopolyploid.

The impact of genetical diploidization on a raw allopolyploid needs little stressing. A single-step change that could confer regular chromosomal pairing and a resultant rise in fertility would have such an evolutionary potential that it would become rapidly selected to fixation.

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STATISTICS

A Combinatorial Assignment Problem

In an establishment, there are $v = nk$ officers, r departments and k types of jobs in each department. Each officer has to be assigned a job in each department such that in any department there are equal numbers of officers in different jobs and any two officers have common jobs in exactly λ departments.

It may be seen that the combinatorial problem is the same as that of a resolvable balanced incomplete block design for $v = nk$ varieties, r replications and k plots per block[1]. We have to identify varieties with officers, replications with departments, and blocks in a replication with jobs in a department. We have the obvious identity $\lambda(v-1) = r(k-1)$.

An important case is when $n = k$. The question arises: What is the smallest value of λ for which the problem admits a solution for any given number k? We thus have a classification of numbers by this minimum λ.

When k is a prime or a prime power it is known that $(k-1)$ mutually orthogonal Latin (Euler) squares exist[2,3]. Let the cells of a $k \times k$ square be taken as officers, the rows as different jobs in the first department, the columns as different jobs in the second department and the letters of the ith Latin square as different jobs in the $(i + 2)$th department. This provides an assignment of jobs to the officers such that any two officers have a common job only in one department (by virtue of the orthogonal property of the Latin squares). Thus in this case $\lambda = 1$, and therefore all primes and prime powers are of type 1.

For $k = 6$, which does not admit even two orthogonal squares, the solution is impossible with $\lambda = 1$. I have been able to find a solution with the next number $\lambda = 2$. Represent the 36 officers by pairs of residues (x, y) mod (5, 7) and an invariant number I. Consider the arrangement in two departments.

Job	Department 1	Department 2
1	01, 06, 12, 15, 23, 24	01, 06, 32, 35, 13, 14
2	11, 16, 22, 25, 33, 34	11, 16, 42, 45, 23, 24
3	21, 26, 32, 35, 43, 44	21, 26, 02, 05, 33, 34
4	31, 36, 42, 45, 03, 04	31, 36, 12, 15, 43, 44
5	41, 46, 02, 05, 13, 14	41, 46, 22, 25, 03, 04
6	00, 10, 20, 30, 40, I	00, 10, 20, 30, 40, I

Adding unity to the second member of each pair (x, y) in the arrangement for any one of the two departments and reducing to mod 7, and keeping I unchanged yields the arrangement for another department. This operation applied to the arrangements for the first two departments leads to the arrangements for two other departments, which by the same operation provides two other arrangements and so on. We thus derive the arrangements for all the fourteen departments. It is not known whether the problem can be solved for any k, with $\lambda = 2$. The next number to investigate is 10, which is unlikely to have 9 mutually orthogonal squares. An arrangement with $\lambda = 4$ is, however, possible. In any event, the open problem is that when a number k is not a prime or a prime power, can we say that it belongs to at least type 2?

The combinatorial problem of assignments is thus a general formulation of a wider variety of problems than the problem of orthogonal Latin squares.

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FORTHCOMING EVENTS

(Meeting marked with an asterisk is open to the public)*

Wednesday, July 5

SOCIETY FOR ENDOCRINOLOGY (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Sir Charles Dodds, F.R.S.: "Rime and Reason in Endocrinology".*

Wednesday, July 5—Friday, July 7

EUROPEAN ORGANIZATION FOR RESEARCH ON FLUORINE AND DENTAL CARIES PREVENTION (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2—Eighth Congress.

Thursday, July 6

BOROUGH POLYTECHNIC (at Borough Road, London, S.E.1) at 10 a.m.—Symposium on "Internal Stresses in Electrolytically Produced Coatings and their Influence on the Properties of the Basis Metals".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH FELLOW (Senior or Junior) IN THE AGRICULTURAL BIOCHEMISTRY DIVISION of the Department of Biological Chemistry, for research on the immunology of the pituitary gonadotrophic hormones—The Secretary, The University, Aberdeen (July 7).

LECTURER (graduate in agricultural chemistry, physics or geology) IN SOIL PHYSICS in the Department of Agricultural Sciences at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (July 8).

RESEARCH ASSISTANT (graduate in agricultural chemistry, biochemistry or chemistry) to Prof. E. G. Hallsworth at the School of Agriculture, Sutton Bonington, for work on the nutrition and biochemistry of the Leguminosae—The Registrar, The University, Nottingham (July 8).

SENIOR LECTURER (male, British, with an appropriate honours degree) IN APPLIED MECHANICS at R.N. College, Greenwich, London, S.E.10, for duties which include lecturing and conducting laboratory work to degree standard and postgraduate teaching—The Director (P), Education Department, Admiralty, London, S.W.1 (July 10).

UNIVERSITY LECTURER IN MATHEMATICAL STATISTICS—The Secretary of the Appointments Committee of the Faculty of Mathematics, The Arts School, Bene't Street, Cambridge (July 12).

CHAIR OF MATHEMATICS—The Registrar, University College of Sussex, Stanmer House, Stanmer, Brighton (July 15).

EXTRA-MURAL TUTOR IN SOCIAL ANTHROPOLOGY or related fields—The Registrar, University College, Singleton Park, Swansea (July 15).

VOKES RESEARCH FELLOW (preferably with a special interest in fluids or elastic theory) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary and Registrar, The University, Southampton (July 15).

HEAD (with high qualifications in botany or zoology and experience in teaching to university degree level and in research) OF THE DEPARTMENT OF BOTANY AND ZOOLOGY (Grade V)—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 17).

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY, to carry out research in the carotenoid field in collaboration with Prof. B. C. L. Weedon—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 17).

RESEARCH FELLOW or ASSISTANT LECTURER IN MATHEMATICS—The Secretary, Royal College of Science and Technology, Glasgow (July 17).

RESEARCH ASSOCIATE (graduate in mathematics or physics), to perform theoretical work on semiconductors, as a member of a small group IN THE DEPARTMENT OF APPLIED MATHEMATICS—Prof. P. T. Landsberg, Department of Applied Mathematics, University College, Cardiff (July 20).

LECTURER (physicist, chemist, or glass technologist, preferably with some industrial experience) IN THE DEPARTMENT OF GLASS TECHNOLOGY—The Registrar, The University, Sheffield (July 22).

CHEMIST (with a degree in chemistry or equivalent qualification, and preferably experience in pesticide analysis) IN THE PLANT PROTECTIVE CHEMISTRY SECTION, to assist in studies of pesticides in fruit culture—The Secretary, East Malling Research Station, Maidstone, Kent (July 24).

SENIOR LECTURER or LECTURER (preferably with previous research experience (theoretical or experimental) in upper atmospheric physics and in palæomagnetism or an allied subject) IN PHYSICS; a LECTURER IN GEOGRAPHY; and a LECTURER IN MATHEMATICS at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 26).

PROTOR MEMORIAL FELLOW (science or technology graduate with postgraduate research experience) IN THE DEPARTMENT OF LEATHER INDUSTRIES, for research on a subject pertaining to the leather or allied industries—The Registrar, The University, Leeds 2 (July 28).

CHAIR OF THEORETICAL PHYSICS; and SENIOR LECTURERS and LECTURERS IN ANATOMY, BIOCHEMISTRY, CHEMISTRY, ENGINEERING, ETHOLOGY AND VERTEBRATE PALÆONTOLOGY, MATHEMATICS, PHYSICS, and PHILOSOPHY at Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 31).

LECTURER or SENIOR LECTURER IN ZOOLOGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 31).

RESEARCH FELLOWS AND STUDENTS IN THE PHYSICS DEPARTMENT, to work in the surface physics group—The Secretary and Registrar, The University, Southampton (August 1).

SENIOR LECTURER or LECTURER IN PARASITOLOGY; and a SENIOR LECTURER or LECTURER (with a special interest in hæmatology or clinical chemistry) IN PATHOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 12).

READER IN VETERINARY MEDICINE at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 22).

CHAIR OF AGRICULTURAL BOTANY in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 23).

CHAIR OF SOIL SCIENCE in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 23).

AGRICULTURAL OFFICER (male, aged 25–45, with a degree in agriculture or a related subject, and experience in tropical agriculture) in Gambia, for general agricultural extension work—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/12/03.

ASSISTANT LECTURER (preferably with a knowledge of elementary virology and/or micology, and some teaching experience) IN MICROBIOLOGY in the Department of Health Education—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

DEMONSTRATOR IN PHYSICS—The Registrar, The University, Nottingham.

LECTURER (graduate at present engaged in industry, research or teaching) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, West Ham College of Technology, to teach either telecommunications or measurements to full-time students preparing for the University of London Internal B.Sc.(Eng.) Degree in Electrical Engineering—Chief Education Officer, Education Department, 95 The Grove, Stratford, London, E.15.

LECTURER (Grade I) IN THEORETICAL PHYSICS; a LECTURER (Grade I) IN PHYSICS; and a LECTURER (Grade II) IN PHYSICS—The Registrar (Room 22, O.R.B.), The University, Reading.

LECTURER (graduate of a British university, teaching experience, and preferably qualified to undertake some teaching of biology) IN PHYSIOLOGY in the Department of Health Education—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

MINERALOGIST (preferably under 35, with a good honours degree in geology, with emphasis on mineralogy and preferably experience of spectrograph and X-ray apparatus) with the Government of Tanganyika, to be engaged in the identification of minerals—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. C1/BCD105/8/04.

RESEARCH ASSISTANTS (2), for work with Prof. R. M. Barrer on the physical and hydrothermal chemistry of soil-forming minerals—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

RESEARCH STUDENTS IN THE DEPARTMENT OF CHEMICAL ENGINEERING, MECHANICAL ENGINEERING, and one other Department—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 17/G.

SENIOR LECTURER (graduate of a British University) IN BIOCHEMISTRY—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

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